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FURTHER STUDIES OF THE MORPHOLOGY OF *Treponema pallidum* UNDER THE ELECTRON MICROSCOPE*

BY

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In previous papers (Ovčinnikov and Delektorskij, 1963, 1966a, b, c), we presented the results of research on the morphology of *Treponema pallidum*. The present paper contains further material on this subject. We are unable to give a definite explanation of the functional significance of a number of morphological findings, but shall nevertheless discuss these findings since the structures observed have not previously been described in the literature. Further information is also given on the *structure* of the fibrillar system of the treponemes. Particular attention is given to the shedding of "termina bodies". Some data are also given concerning the structure of *Leptospira* and *Borrelia*.

The methods of work were the same as those already described. In electron-microscope studies of preparations processed by the negative-contrast method it sometimes happens that the contrast substance does not reach the body of the treponeme. This suggests that the treponeme is surrounded by an envelope or casing, possibly of a mucous nature, which hinders penetration of the contrast mass into the treponeme. This is clearly visible in Figs 1 and 2 and it can be said with great confidence that the body of the treponeme is surrounded by quite a wide "mucoid casing" (CO). Depending on conditions that have so far not been elucidated, the casing may be thicker or thinner. It is possibly protective in function and may not be a constant feature. It occurs mainly when the treponeme is exposed to unfavourable chemical factors. A similar casing (CO) is also found in leptospirae (Fig. 3).

It is an interesting question whether both ends of a treponeme are identical in structure; until now we had assumed that they were, but recent results have convinced us that they are not always so.

Fig. 4 presents a general view of a treponeme of the Nichols strain from a 7-day orchitis. The difference in the structure of the two ends of the treponeme is clearly visible and becomes particularly obvious in the detailed photographs (A and B) taken at high magnifications. One end of the treponeme (Fig. 4A) is elongated and rounded and just in front of it there is a spongy mass (O). A little distance from the end are the basal granules (B) with fibrils (F) attached. The opposite end of the treponeme is thinner (4B); it also contains a spongy mass (O) and fibrils (F) are also attached to the basal granules (B), but in front of the sector in which the last fibril is inserted there is a dilatation in the shape of a cavity, which gradually narrows and from this cavity arise sausage-shaped structures. Similar structures are included in the elongated portion and surrounded by a spongy mass. They are bent and have rounded ends of different sizes. We have difficulty in conceiving the significance of this terminal structure. Possibly one end is the "cephalic" end and the other the "excretory" end, but this is only a supposition. It is also possible that the terminal structures are connected in some way with the nature of division.

It should be noted that we found a difference in the structure of the two ends of the treponeme in a

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number of other specimens of the same strain, and that, while one end was more or less identical in every case and terminated in a spongy mass with basal granules, the opposite end had a different structure. Figs 5, 6, 9, and 10 show the appearance of these terminal structures. They differ somewhat from each other and from the opposite end. A difference in the structure of the two ends of the treponeme can also be seen in cultivated treponemes but not on every occasion (Figs 7 and 8). In our earlier papers we published a number of photographs which indicate the difference between the "head end" and the "excretory" "tail" structure (Ovčinnikov and Delektorskij, 1963, 1966a, b, c).

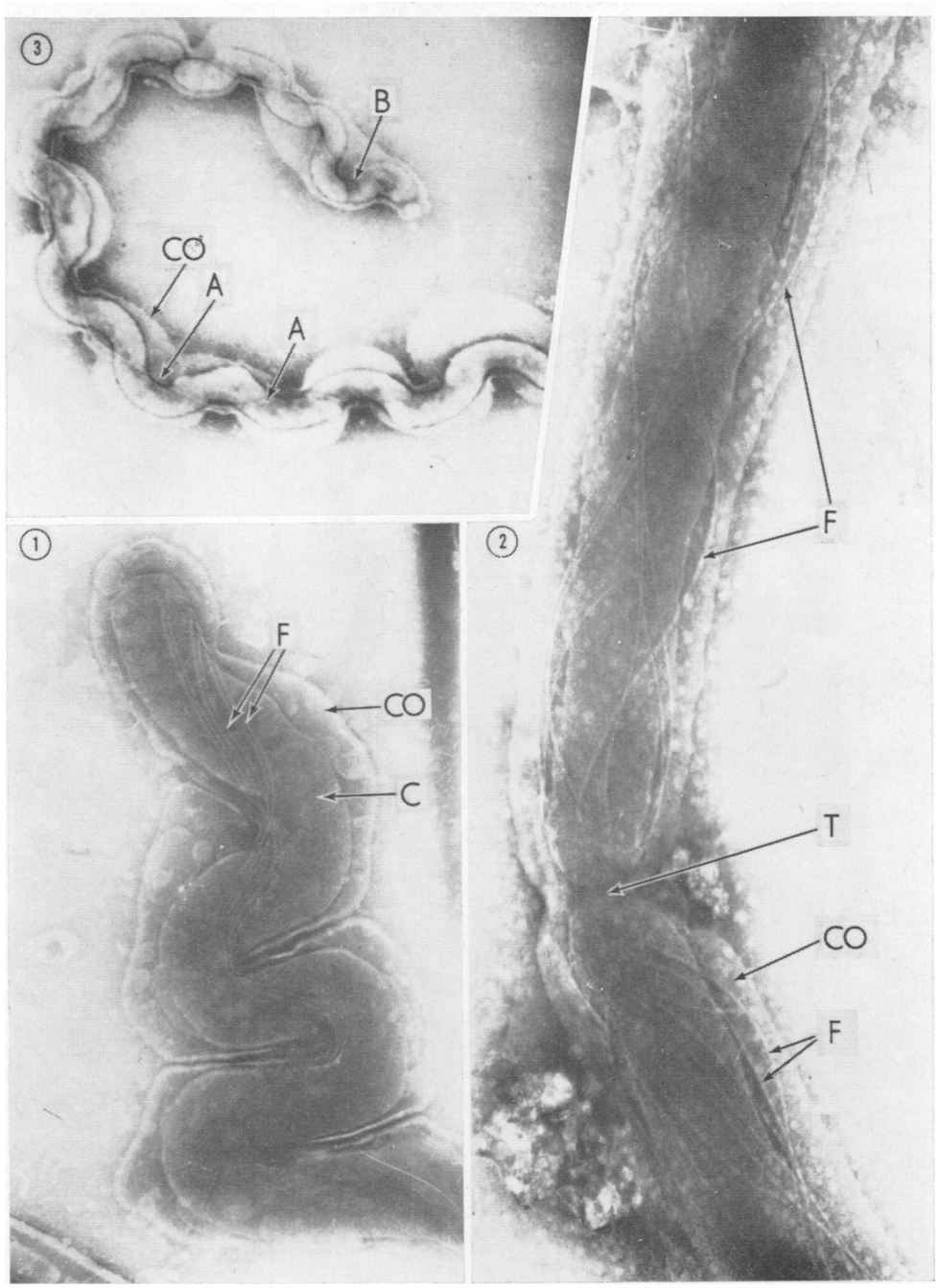
In cultivated treponemes there is an oval or round structure (K) at one end, with basal granules (B) having fibrils inserted in them (F) (Figs 11, 12, 13, 14, 15, 16, 17, and 18). These round structures break off from the treponeme body together with the fibrils at some periods of development. After this structure has broken off, basal granules and new fibrils, which stretch along the whole body of the treponeme, are formed on the part of the end that is left, but this is not always clearly marked. At the opposite end there is no such shedding of structures. The shed terminal structure gradually decreases in volume (Figs 17 and 18) and only the fibrils remain, together with the debris of the terminal structure and what seems to be one basal granule. This is clearly visible for instance in Figs 17 and 59 (B). A similar shedding of a terminal structure together with the axial thread (axostyle) also occurs in leptospirae. Fig. 19 shows the shedding of a terminal structure (K) by *Leptospira biflexa* together with the basal granule and the axostyle (A). It should be noted that leptospirae may possess one azostyle (Fig. 56) or two (Fig. 20A, Fig. 21A). These photographs contradict the statement by Simpson and White (1961) that there is only one axostyle.

While our previous work did not produce extensive evidence on the question of the shedding of the head structure together with the fibrils, we now have abundant electron-microscope material indicating that this occurs not infrequently and is not a sign of breakdown of the treponeme since it is found also in dividing forms. We noted that the head structures, when examined by darkfield, make very active rotatory movements. We find it difficult to explain the causes and object of the shedding of terminal structures together with fibrils: there is perhaps an analogy with the dissemination of seeds provided with various adaptive mechanisms to ensure their distribution.

We stated in our previous papers that *Treponema pallidum* is of uneven thickness throughout its length and that the thickness of various sectors may change when the treponeme moves. This is clearly seen in Fig. 22, where it can also be seen that the two ends differ somewhat in their structure. At one end there is a sort of high "crown" on the snake-head structure. In other Figures (26, 28, 29, 35W) this structure is flatter. Along the length of the treponeme two types of structure attract attention. Some (Fig. 23 and Fig. 32M) are round and not as big as the treponeme in diameter. In most cases their structure is blurred or else it has elongated round inclusions, some of which are covered with a sort of lid or "operculum" (W) under the envelope. Sometimes the operculum (W), which is a lamellar structure, is all that is visible (Figs 25, 26, 27, 28, 29W). In this case the operculum is under the outer envelope (Figs 25, 26, 27). It may take the shape of a process stretched out along the whole of one end of the treponeme and inside it a lumen (H) (Figs 36, 37, 38, and 39) can be seen; it is situated above the entrance to the mesosome. Apparently the purpose of this structure is to act as a valve for the mesosomal contents. The crown-like structure which is situated a little behind the head sector, already described, is likewise a mesosomal valve of some kind. Under it lies a sort of lamellar structure. Possibly this is a mesosome.

KEY TO THE FIGURES

F —fibrils
F″ —second layer of fibrils
C —treponemal body of cytoplasm
CO —outer envelope or "casing"
ME—outer wall
CM—cytoplasmic membrane
B —basal granules
T —site of fission
N —nuclear vacuole
M —mesosome
A —axostyle
R ribosome
U —separate bundle of fibrils
K —terminal structures
W —mesosomal operculum
G —granular treponeme
MCY—common envelope of the cyst
L —light-coloured treponeme
D —dark-coloured treponeme
I —thin treponeme
O —spongy mass
V —mesosomal exit
S —canal
H —lumen
Y —surface protuberance



FIGS 1 and 2.—*Treponema pallidum,* strain Stavropol 7. 7-day growth on Tarozzi medium with rabbit serum. ×36,000. "Casing" clearly visible. In Fig. 2 the casing encloses a dividing form. In both juvenile forms of treponeme new fibrils and basal granules are visible.

FIG. 3.—*Leptospira biflexa.* 2-day growth. ×36,000. "Casing" in leptospirae.

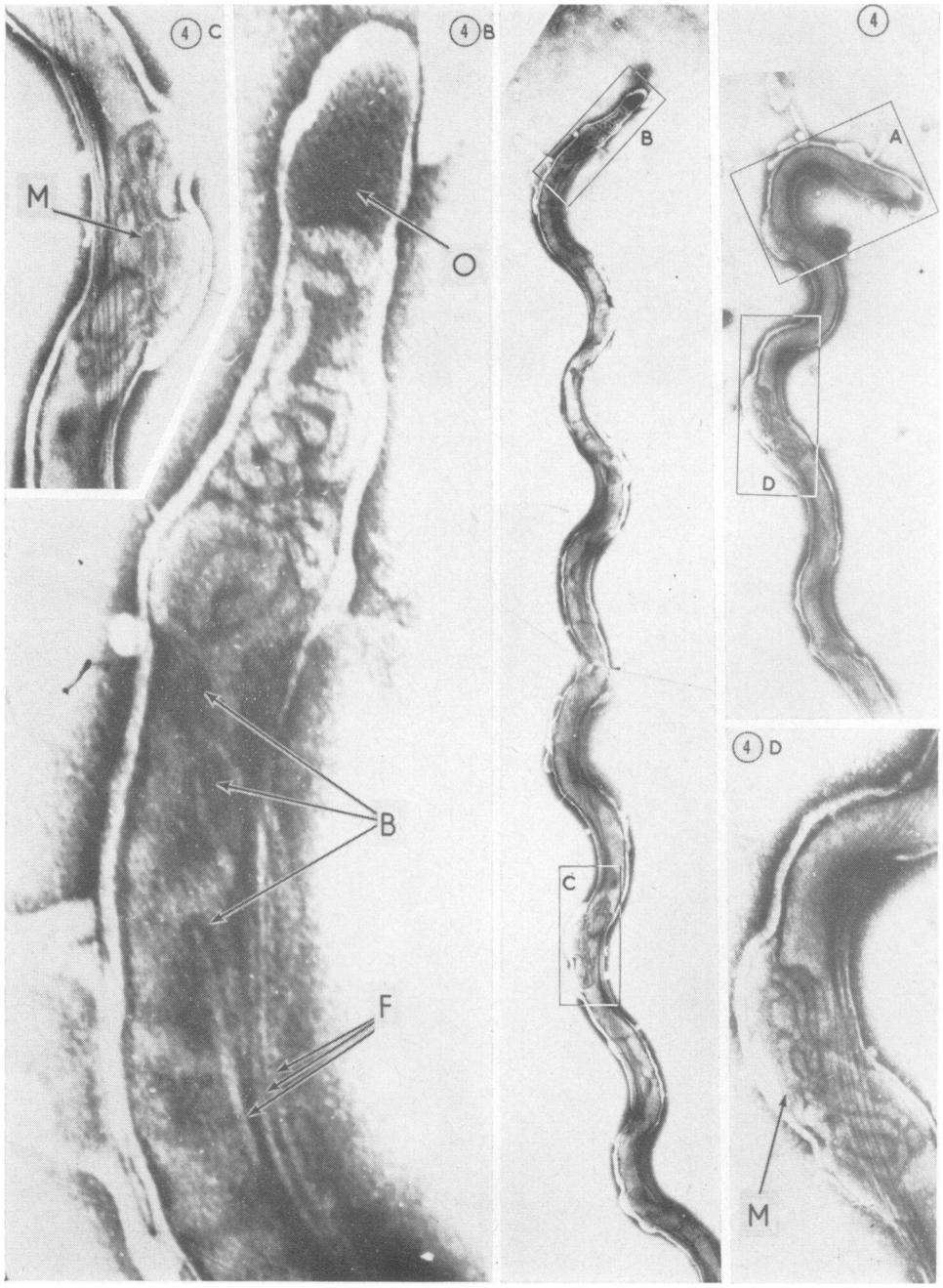
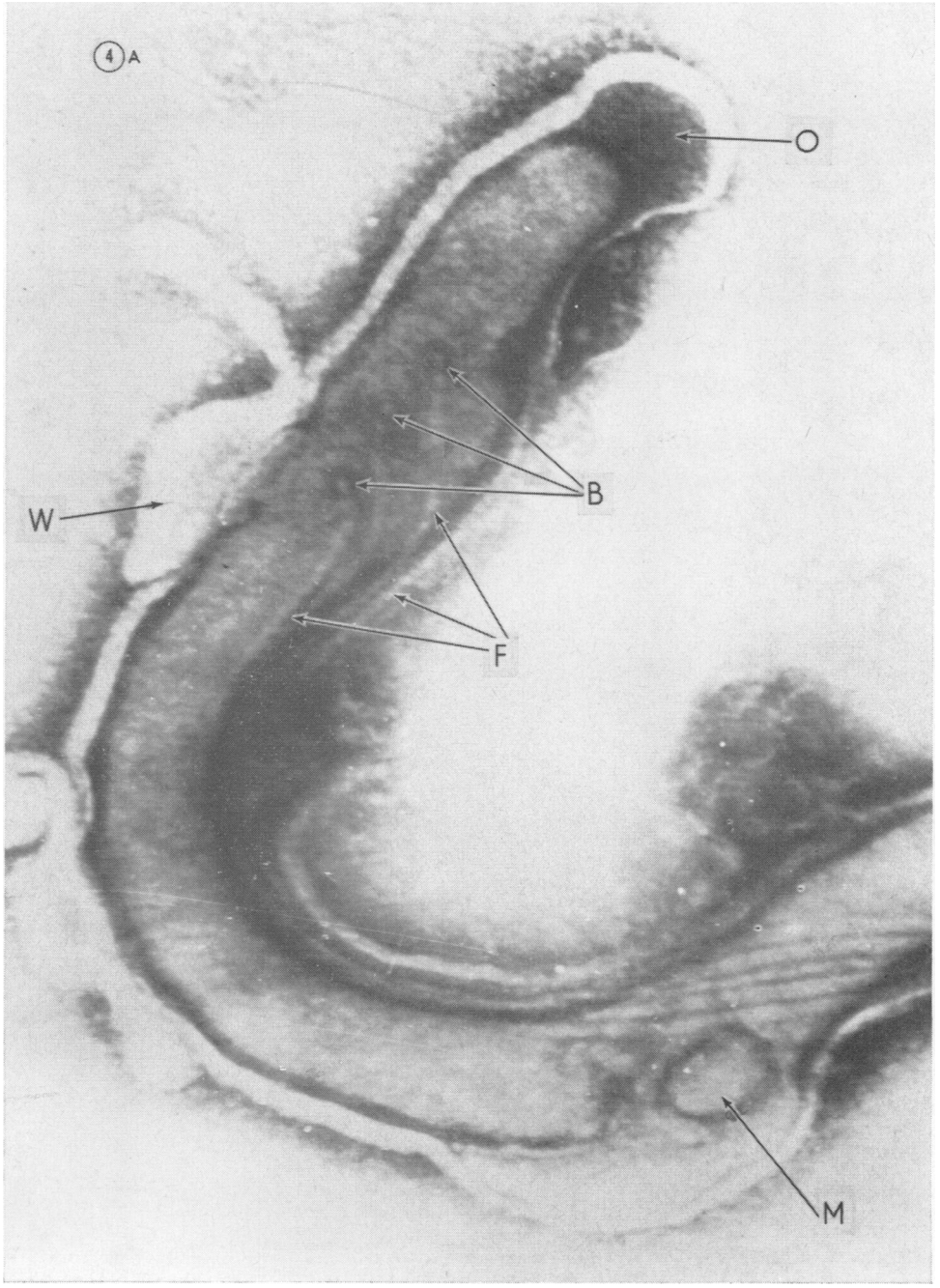
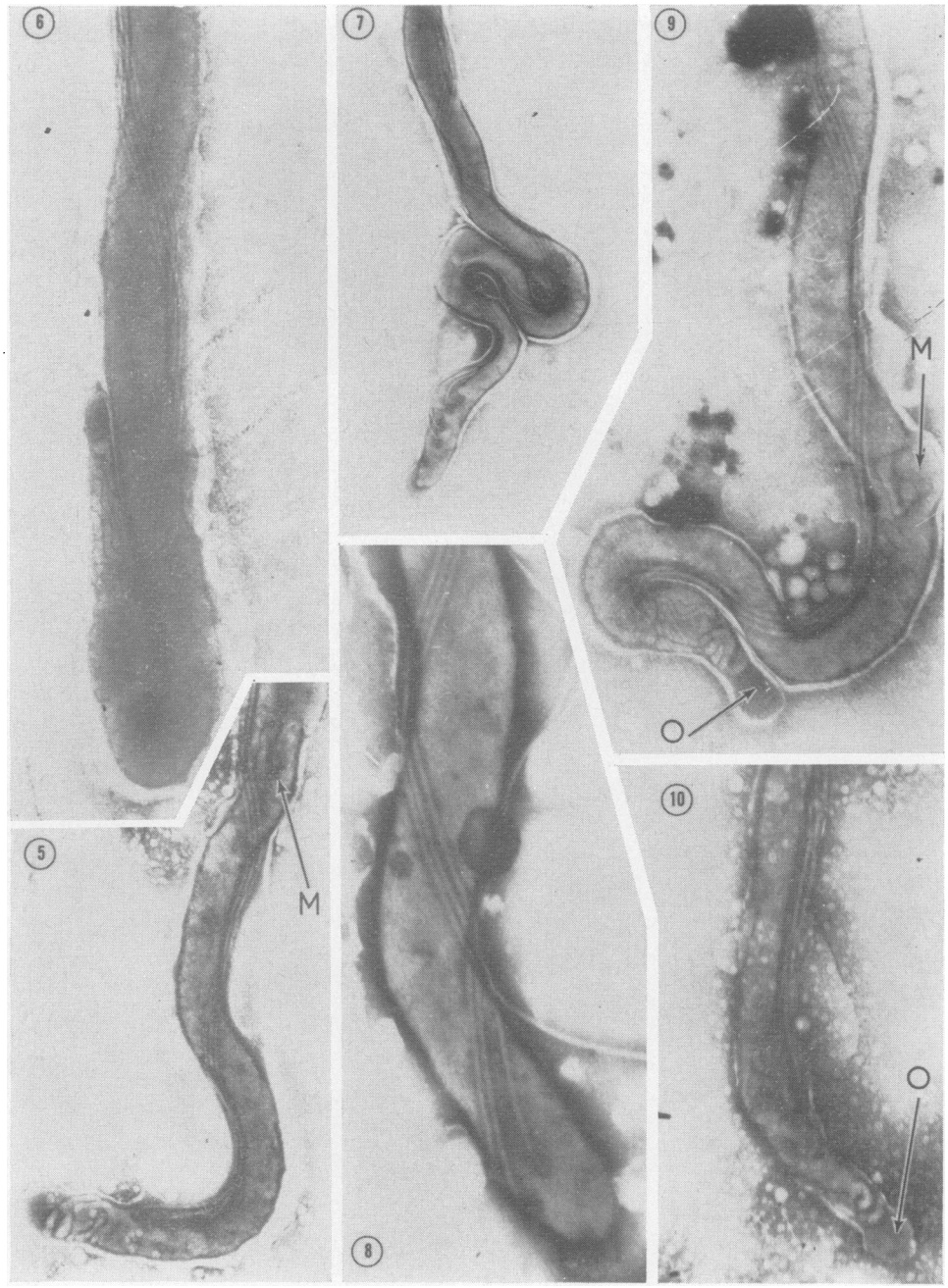


FIG. 4.—*Treponema pallidum*. Nichols strain, from a 7-day orchitis. ×27,000. Different structure of terminal formations. 4A, B, C, and D show details.

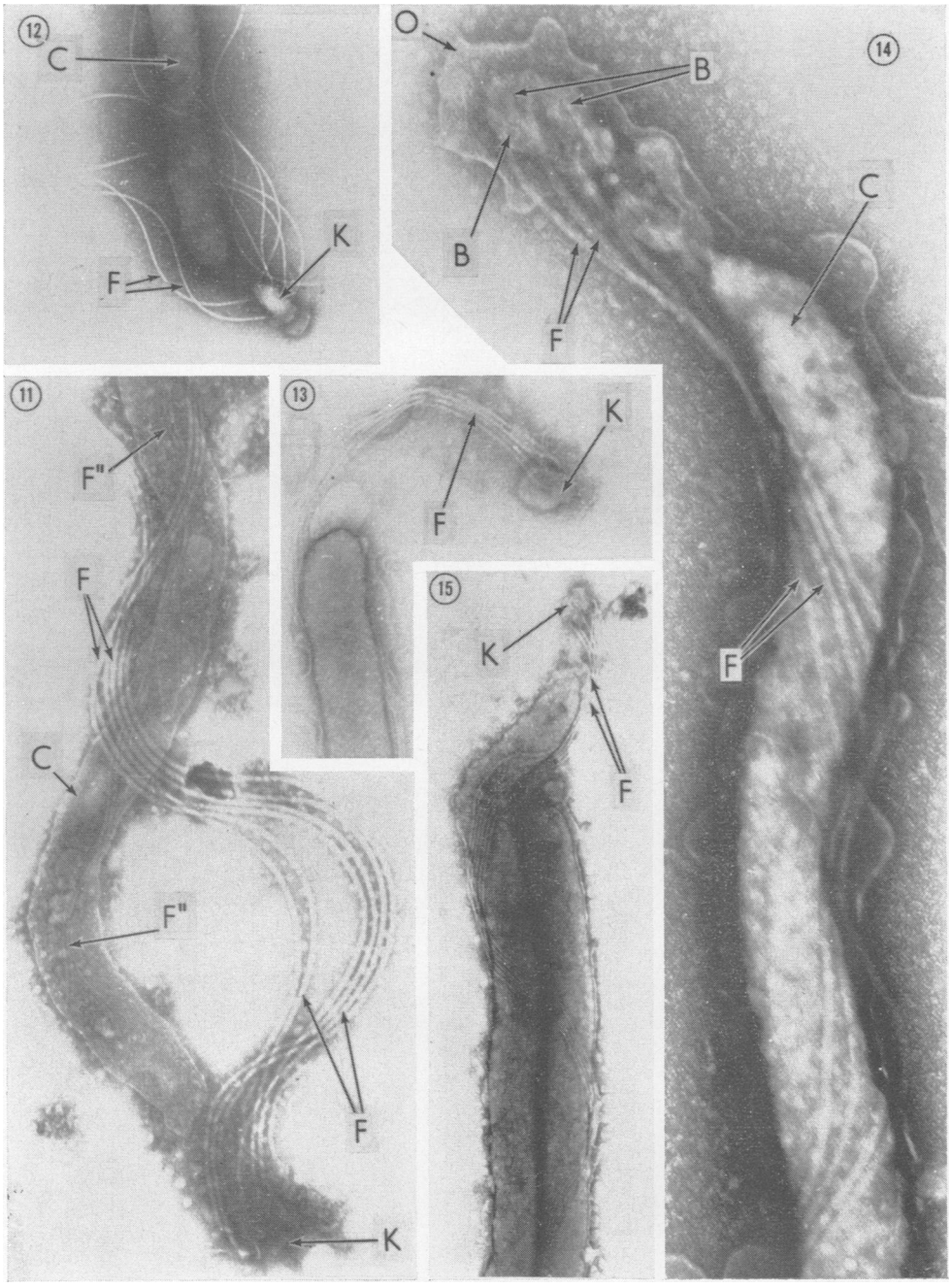

4 A
O
B
W
F
M



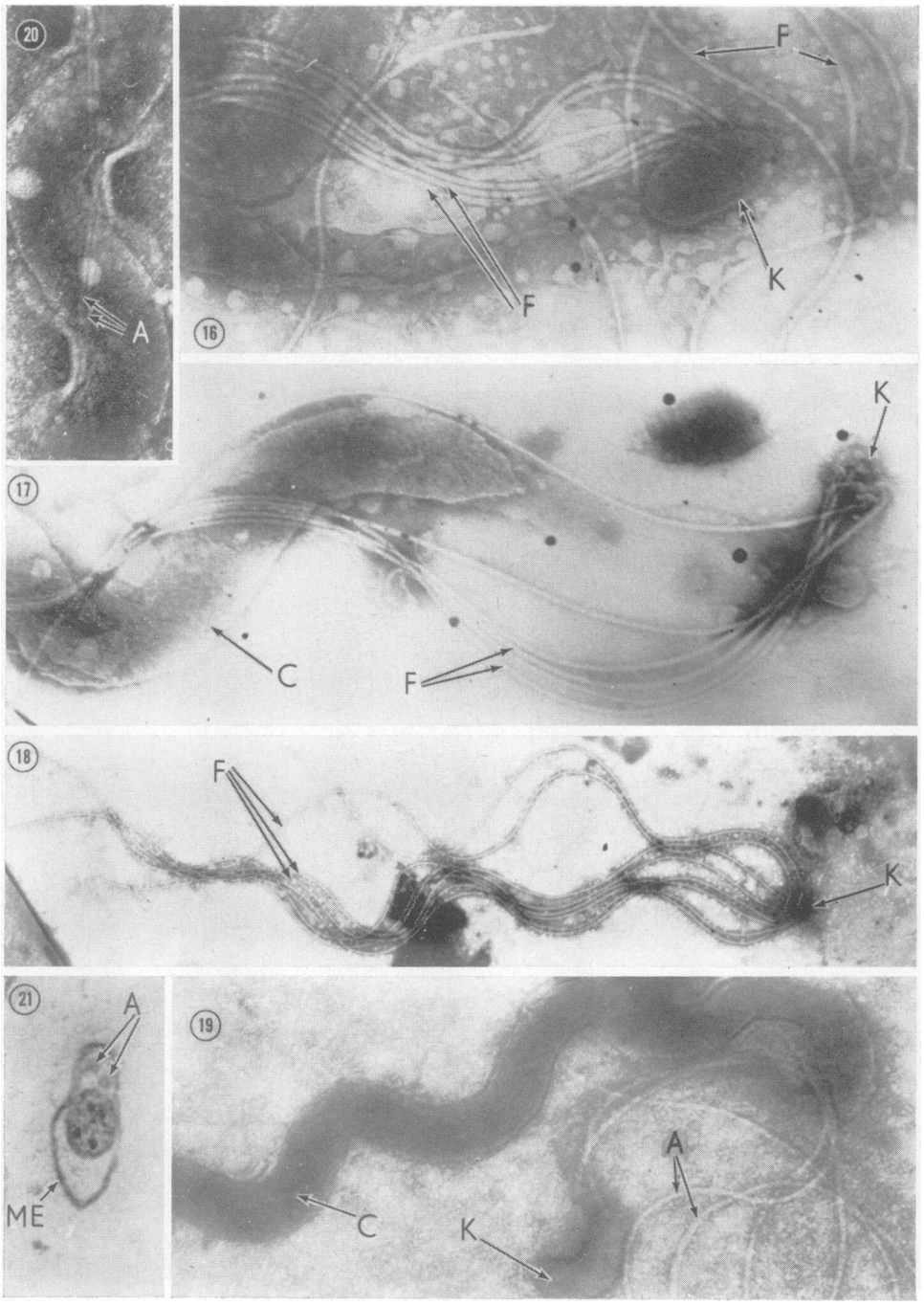
FIGS 5, 6, and 7.—*Treponema pallidum,* Nichols strain. ×27,000. Different structure of terminal bodies.

FIG. 8.—*Treponema pallidum,* strain Kazan 2. 14-day growth. ×27,000.

FIGS 9 and 10.—*Treponema pallidum,* Nichols strain from a 7-day orchitis. ×27,000. Different structure of terminal bodies.



FIGS 11–18.—*Treponema pallidum,* strain 5. 7-day growth. ×27,000. Shedding of terminal structures.



FIGS 19 and 20.—*Leptospira biflexa,* 2-day growth. ×36,000. Fig. 19 shows shedding of a terminal structure by a leptospira. Fig. 20 shows two axial filaments.

FIG. 21.—*Leptospira biflexa,* 21-day growth. Ultrathin section. ×36,000. Two axostyles clearly visible.

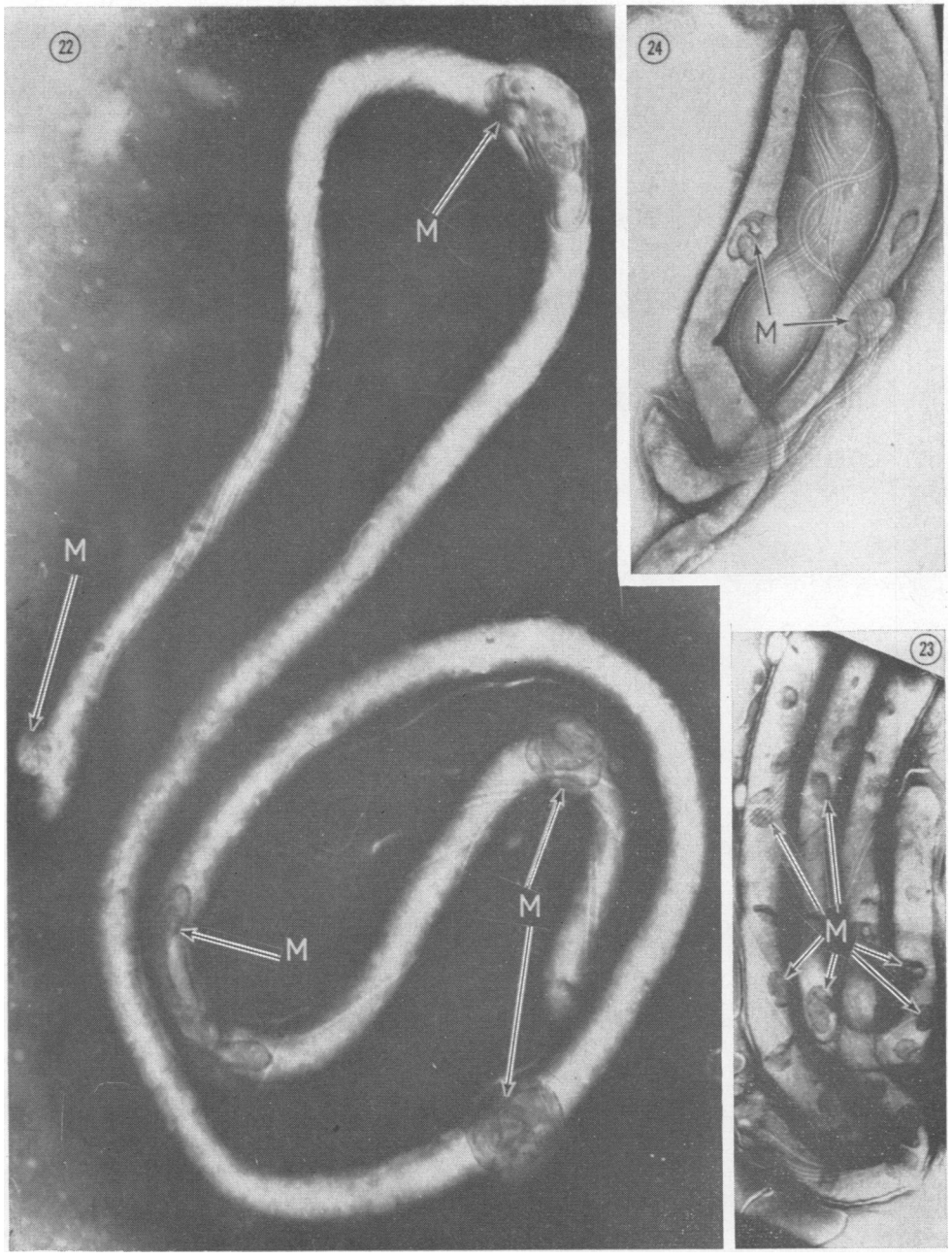
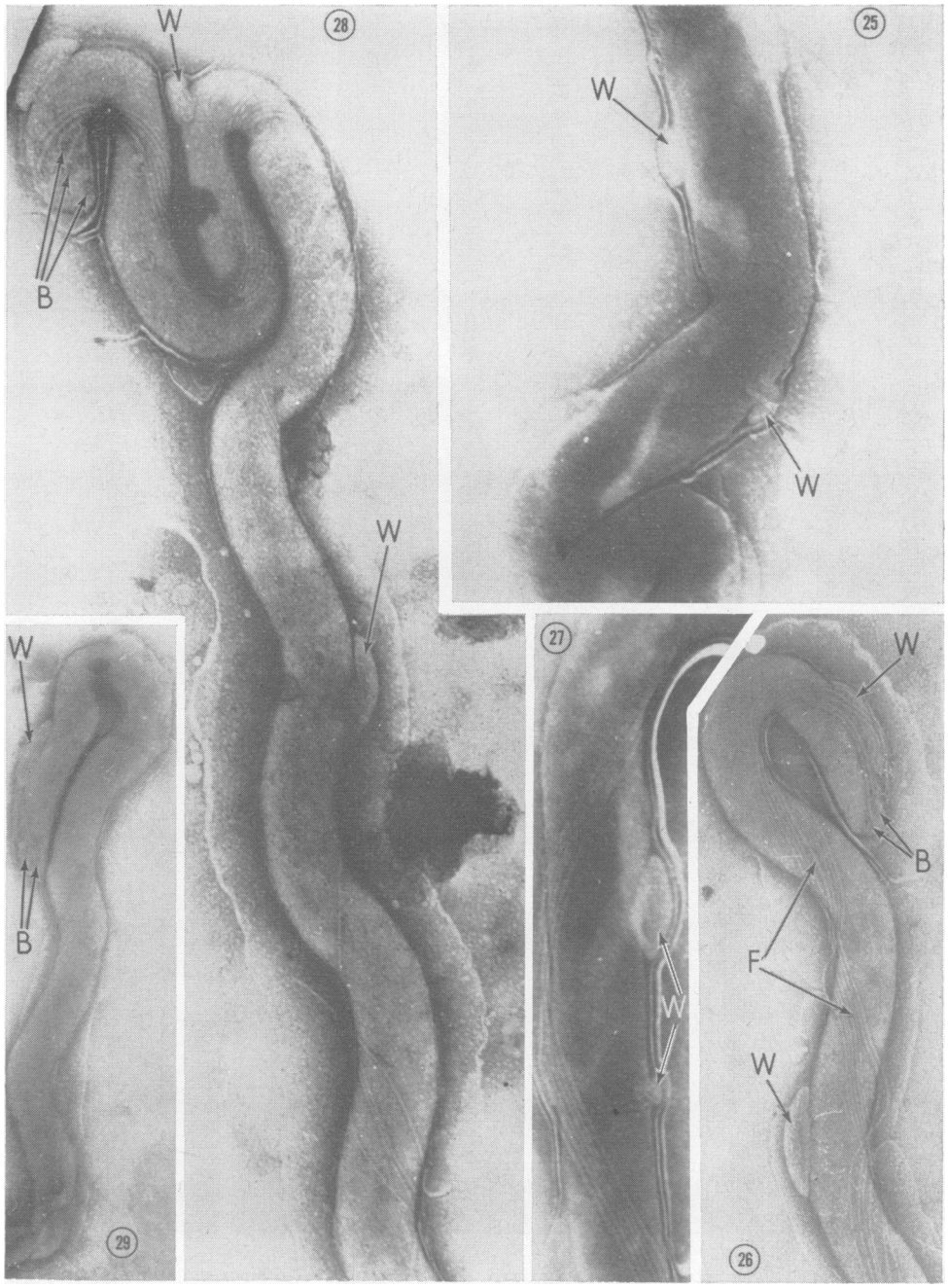
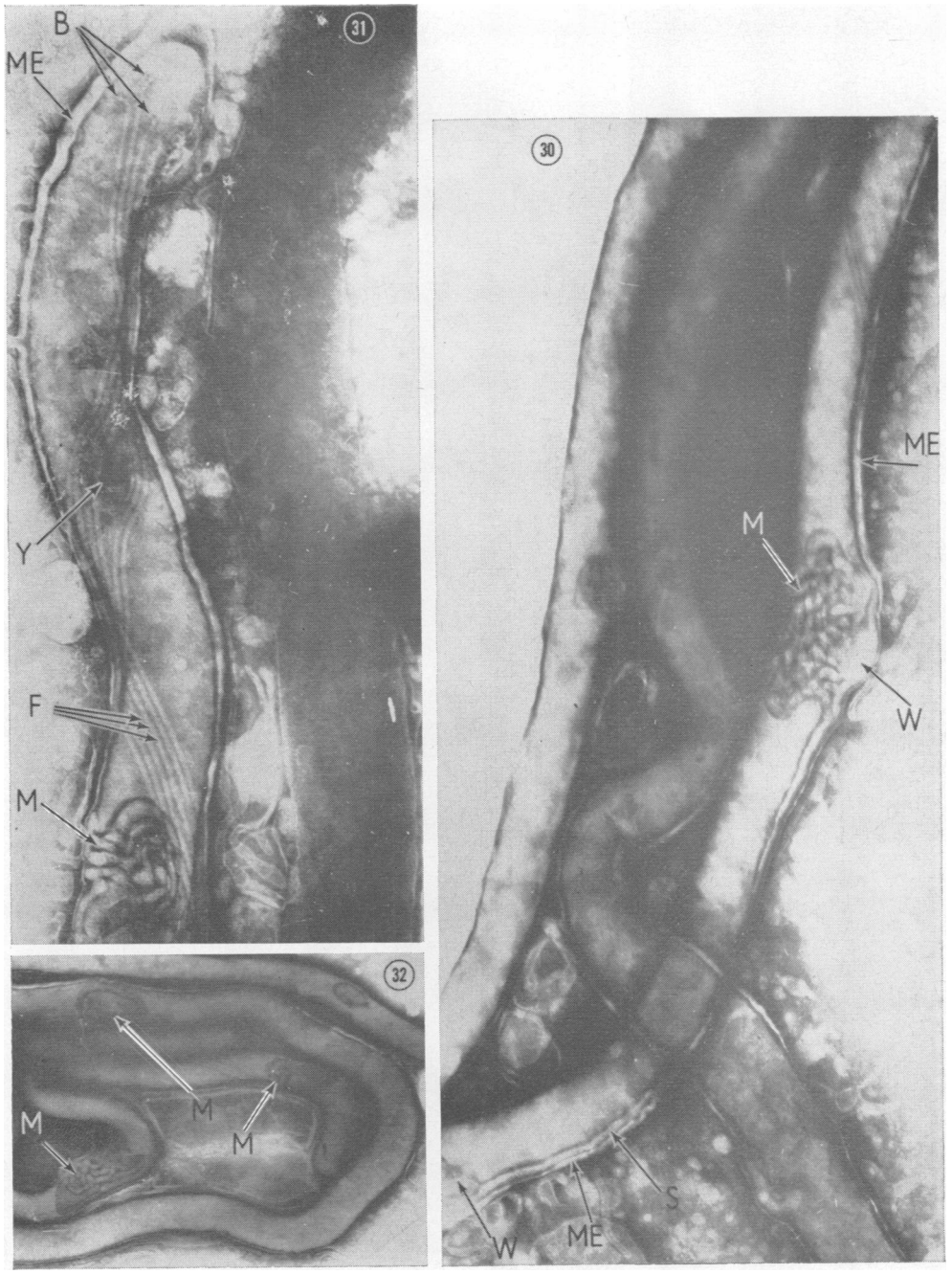


FIG. 22.—*Treponema pallidum*, strain 4. 15-day growth on Tarozzi medium with rabbit serum. ×27,000. Thickness of treponeme and appearance of mesosomes differ in different areas.

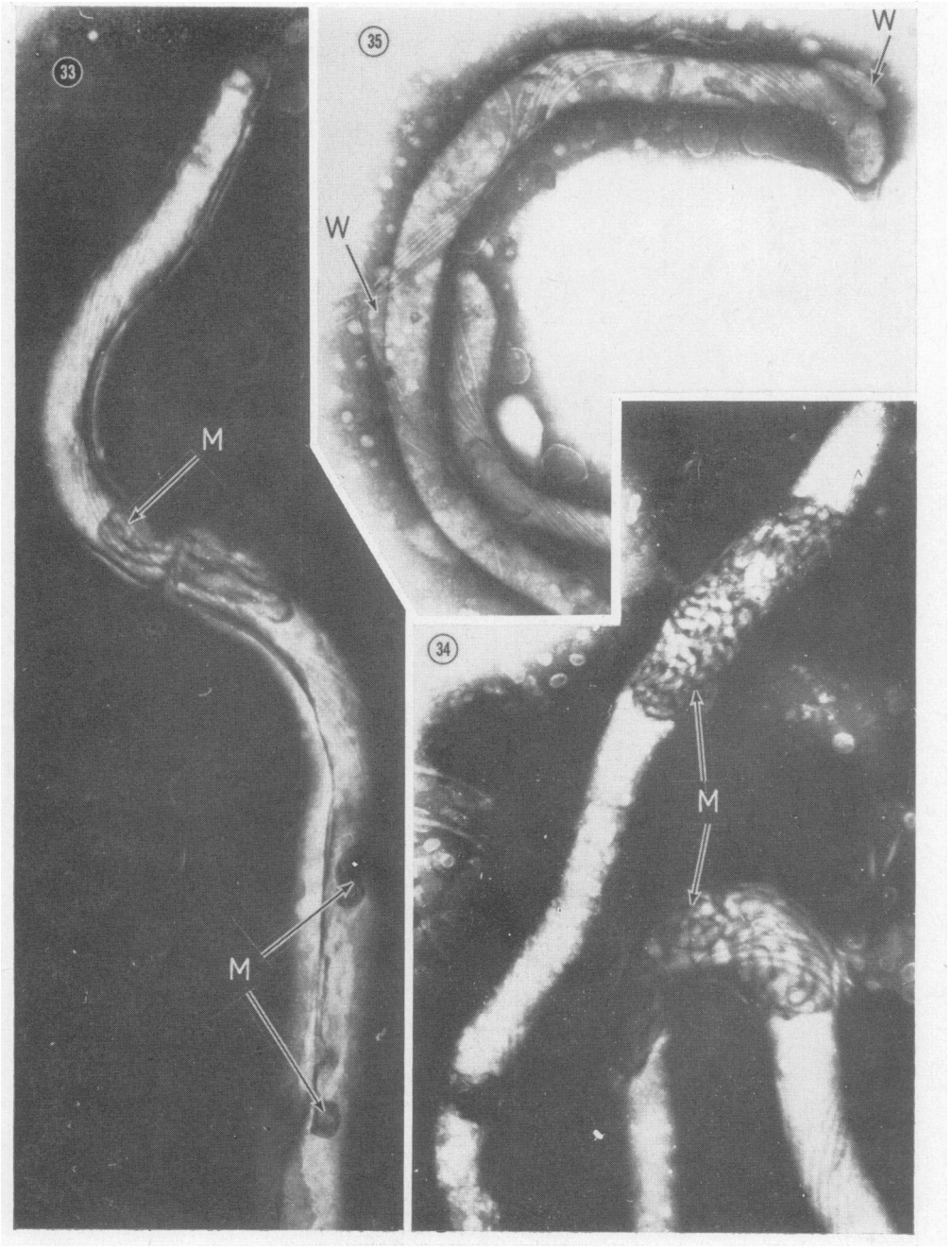
FIGS 23 and 24.—*Treponema pallidum*, strain Stavropol 7. 7-day growth. ×27,000. Mesosomes have a different appearance in different parts of the treponeme.



FIGS 25–29.—*Treponema pallidum*, strain 5. 7-day growth. ×27,000. Mesosomal operculum.



FIGS 30–38.—*Treponema pallidum*, strain 4. 15-day growth. ×27,000. Appearance of mesosomes differs.


33
M
M
35
W
W
34
M

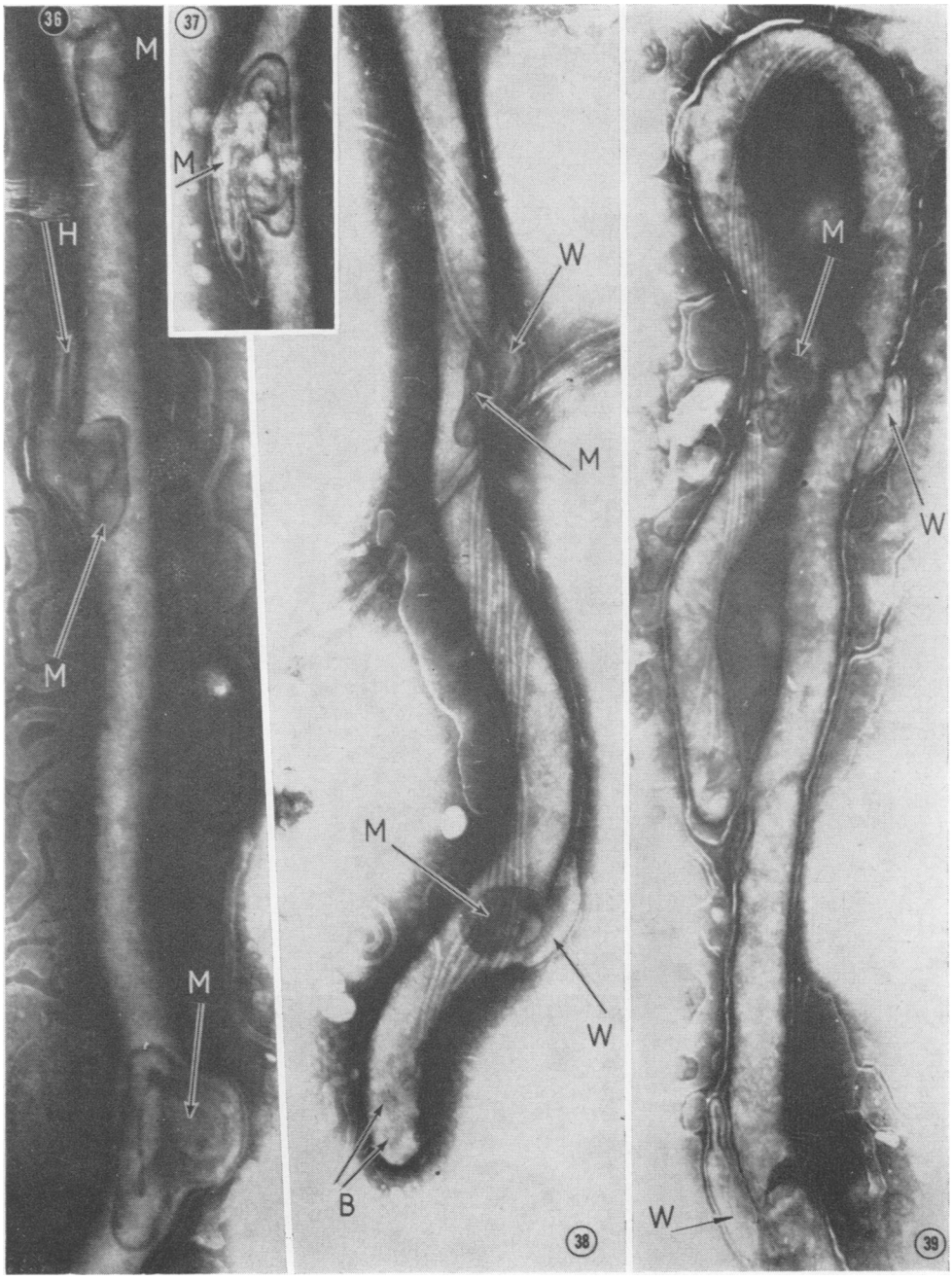
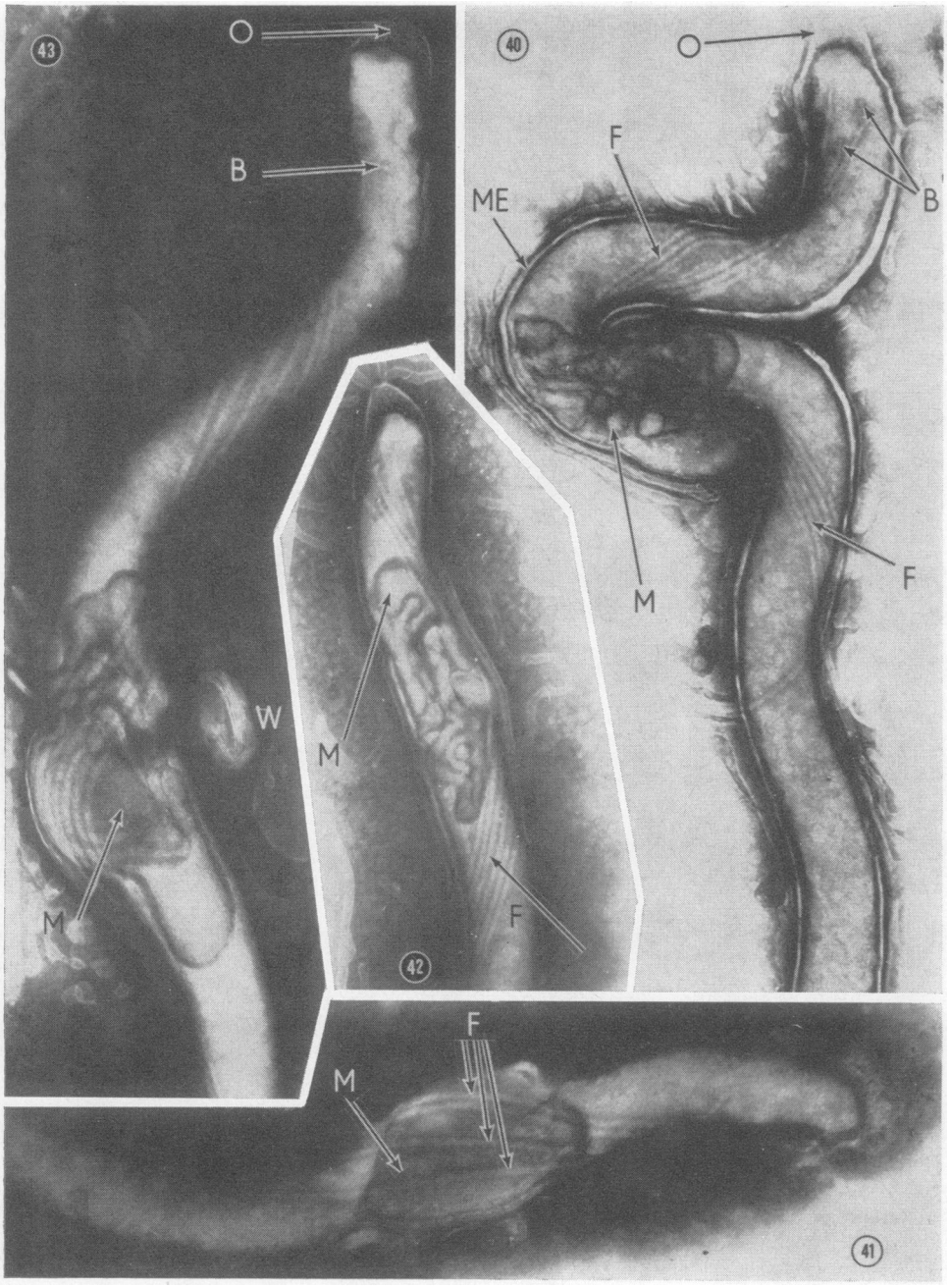


FIG. 39.—*Treponema pallidum*, strain 5. 7-day growth. ×27,000.



FIGS 40–43.—*Treponema pallidum*, strain 4. 15-day growth. ×27,000. Features of mesosomal structure.

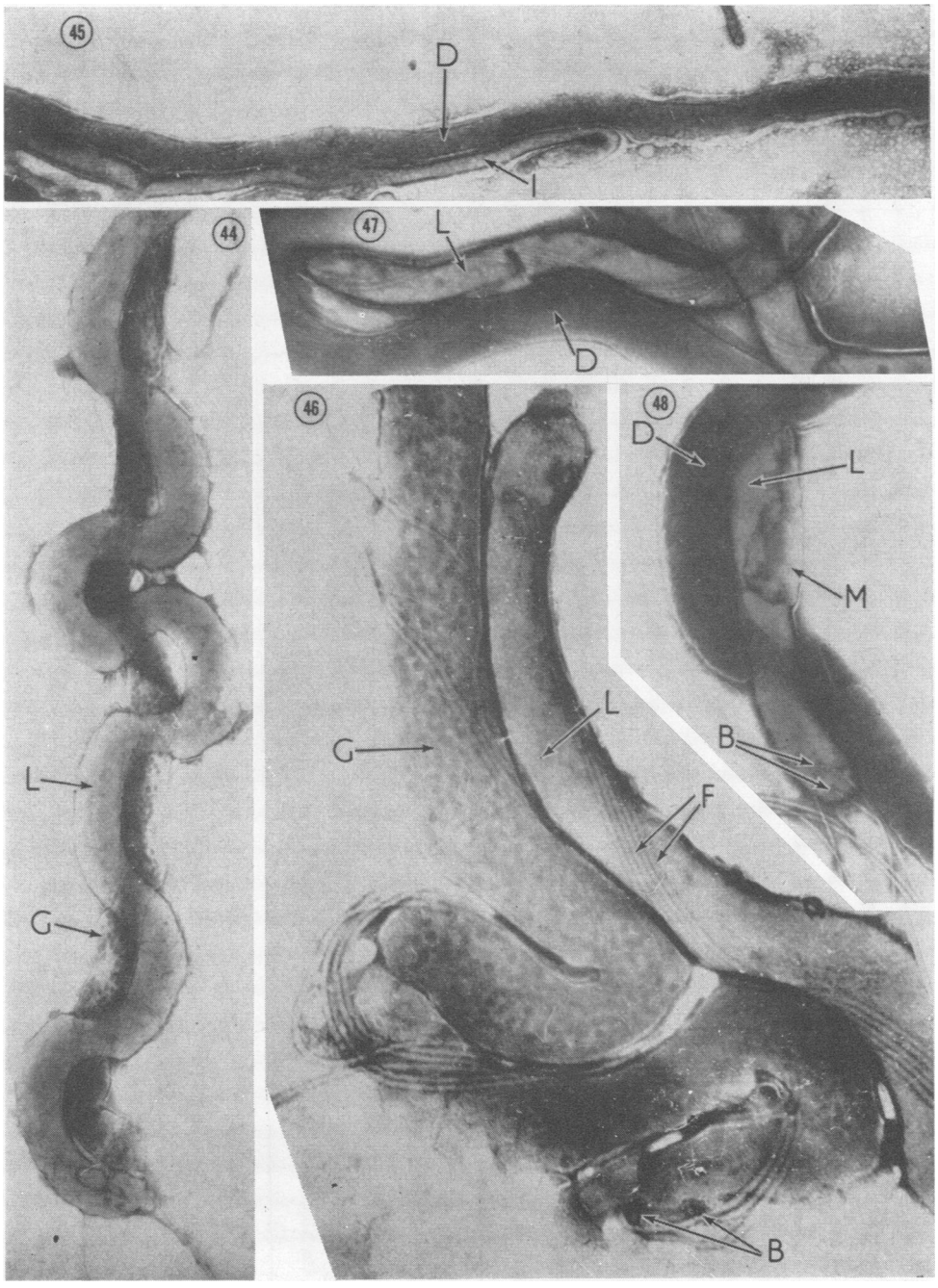
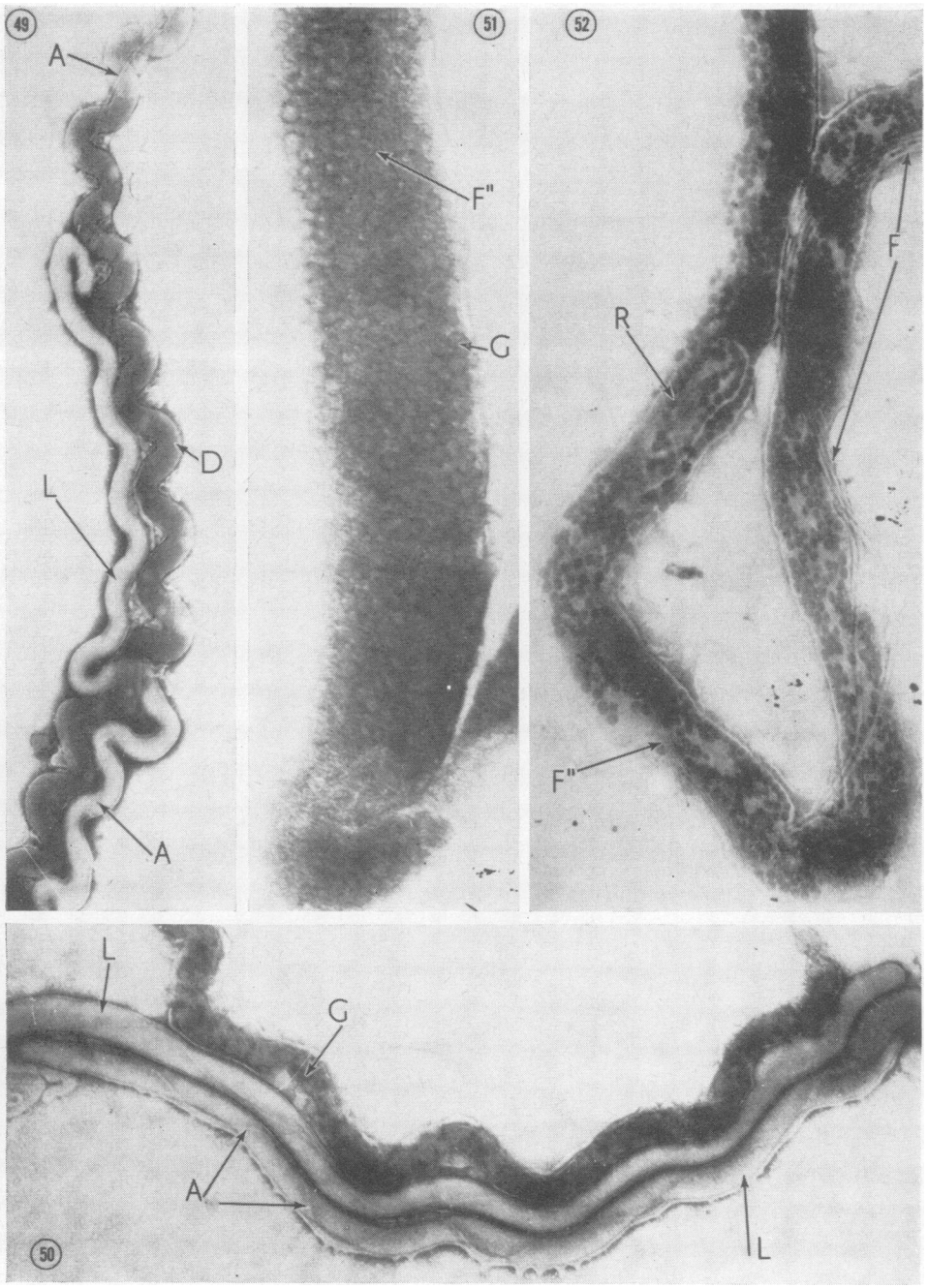


FIG. 44.—*Treponema pallidum*, strain 5. 7-day growth. ×27,000.

FIG 45.—*Treponema pallidum*, Nichols strain, from a 7-day orchitis. Thick and thin treponemes. ×36,000.

FIG. 46.—*Treponema pallidum*, strain 5. 7-day growth on Tarozzi medium with rabbit serum. ×27,000. Granular and homogeneous treponemes.

FIGS 47 and 48.—*Treponema pallidum*, strain 5. 7-day growth. ×27,000. Light and dark treponemes.



FIGS 49 and 50.—*Leptospira biflexa.* 21-day growth. × 27,000. Light and dark structures.

FIGS 51 and 52.—*Treponema pallidum,* strain 5. 7-day growth. × 27,000. Granular treponemes and arrangement of granular areas.

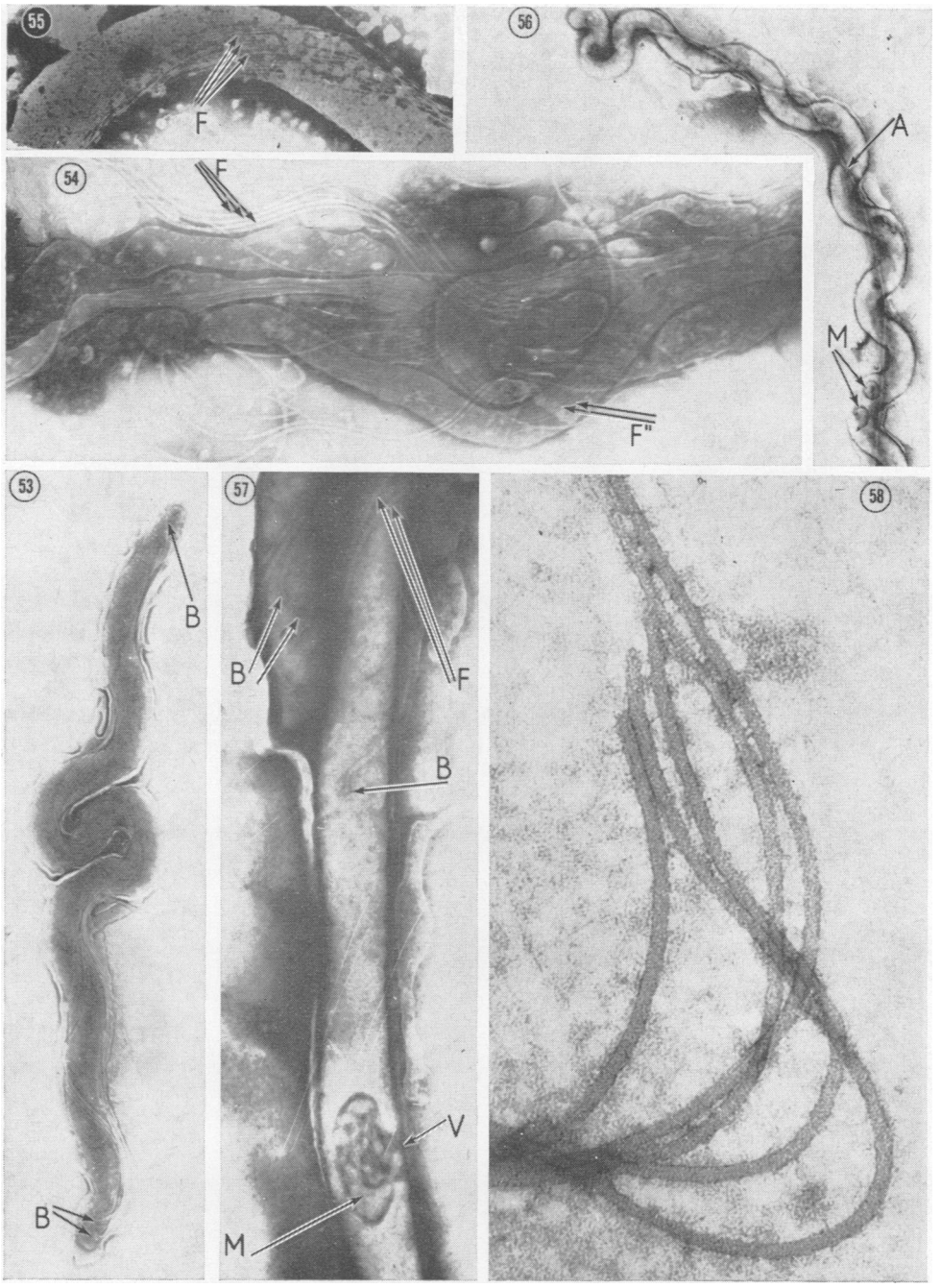


FIG. 53.—Budapest strain of *Treponema pallidum* from a 30-day-old chancre. Large numbers of fibrils in different parts of treponeme. ×36,000.

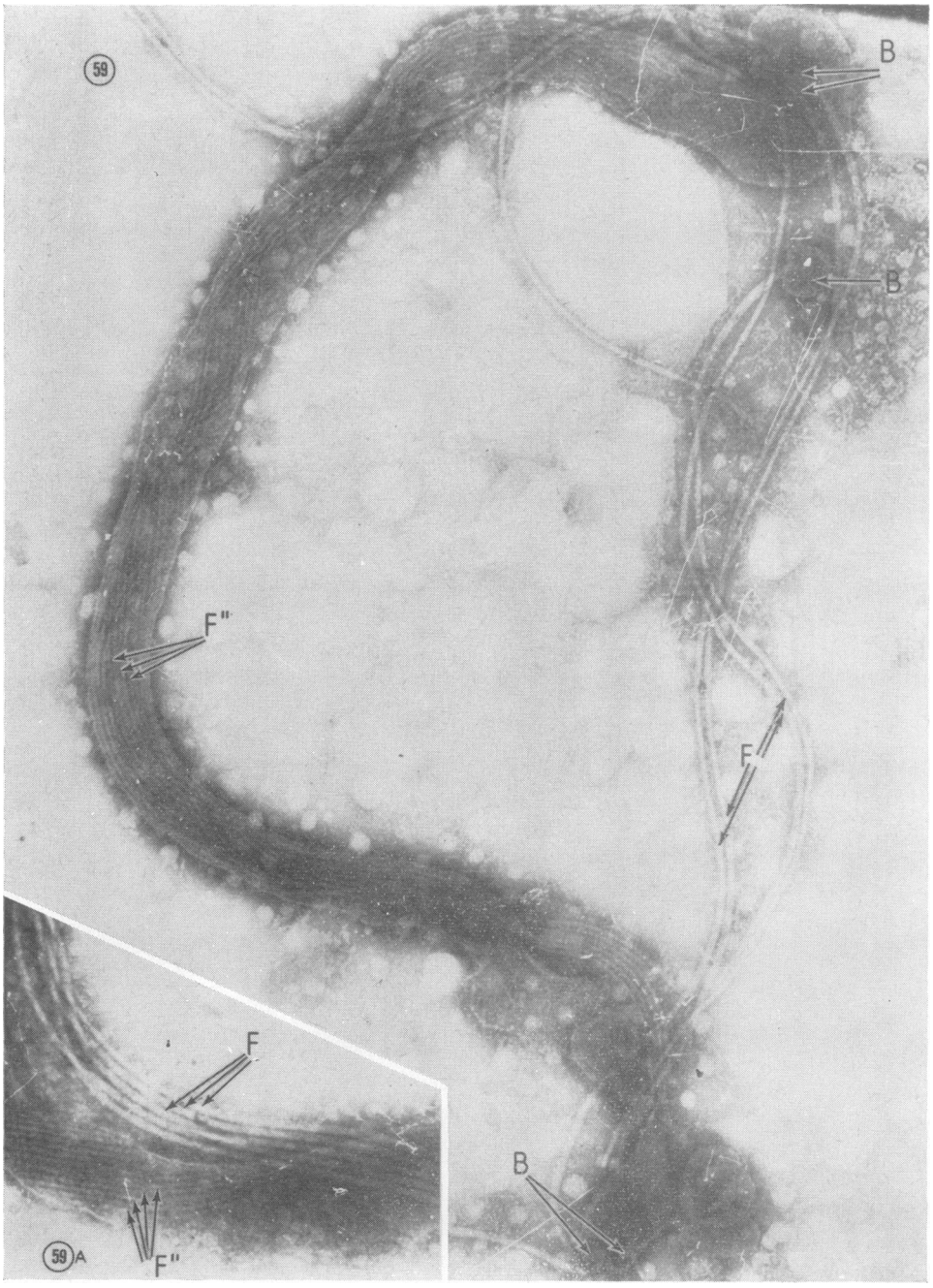
FIG. 54.—*Treponema pallidum*, strain Stavropol 7. 14-day growth. Cryolysis. Differential centrifugation. ×36,000. Breaking off of head structure. Deep-lying fibrillar system.

FIG. 55.—*Borrelia recurrentis caucasica* from the blood serum of a guinea-pig. ×36,000. Fibrils considerably thinner than in treponemes.

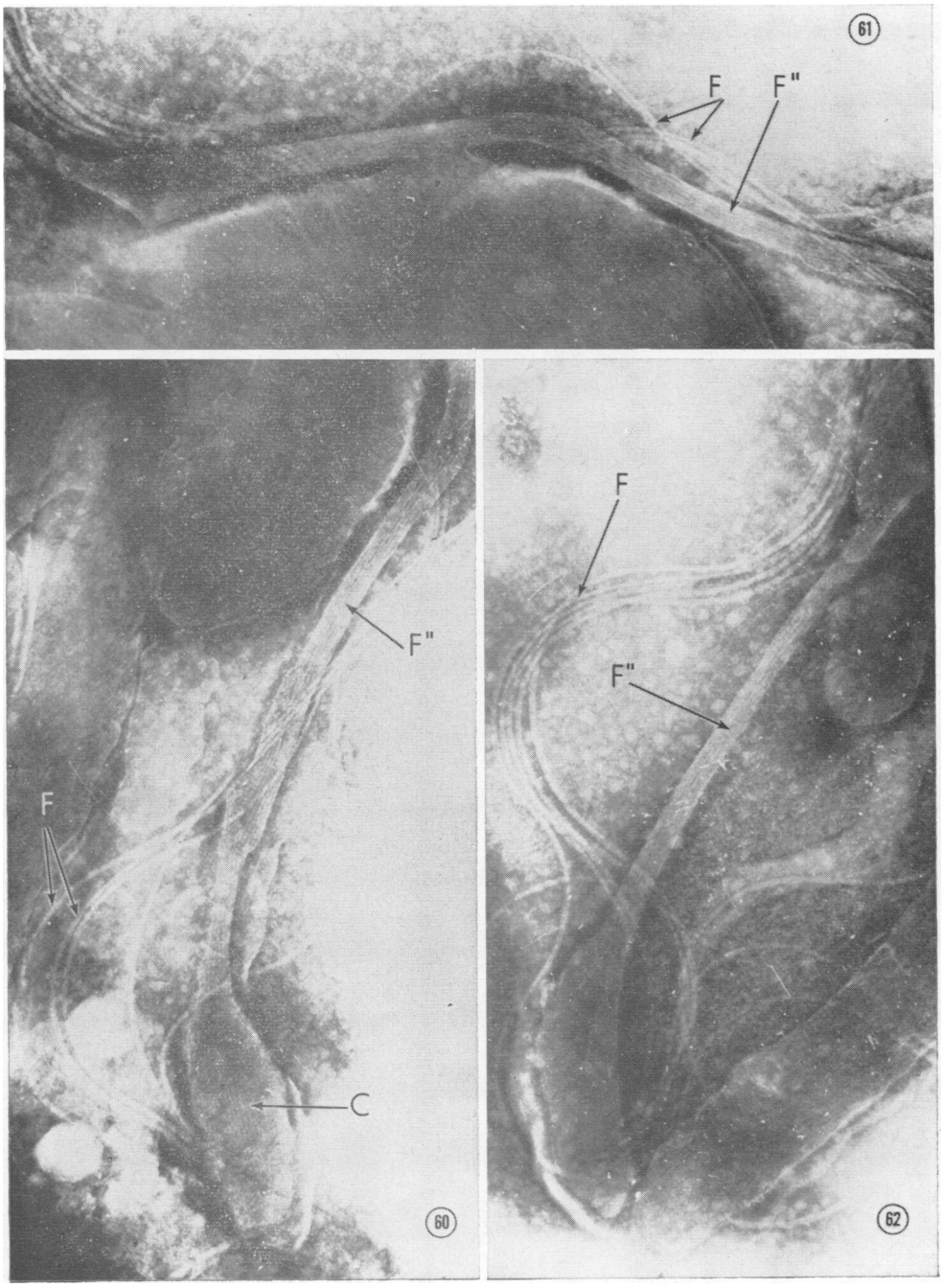
FIG. 56.—*Leptospira biflexa*. 21-day growth. ×27,000. Mesosomes, axial filament, and basal granule clearly visible.

FIG. 57.—*Treponema pallidum*, strain 4. 15-day growth. ×36,000. Basal granule with fibril attached situated in middle of treponeme.

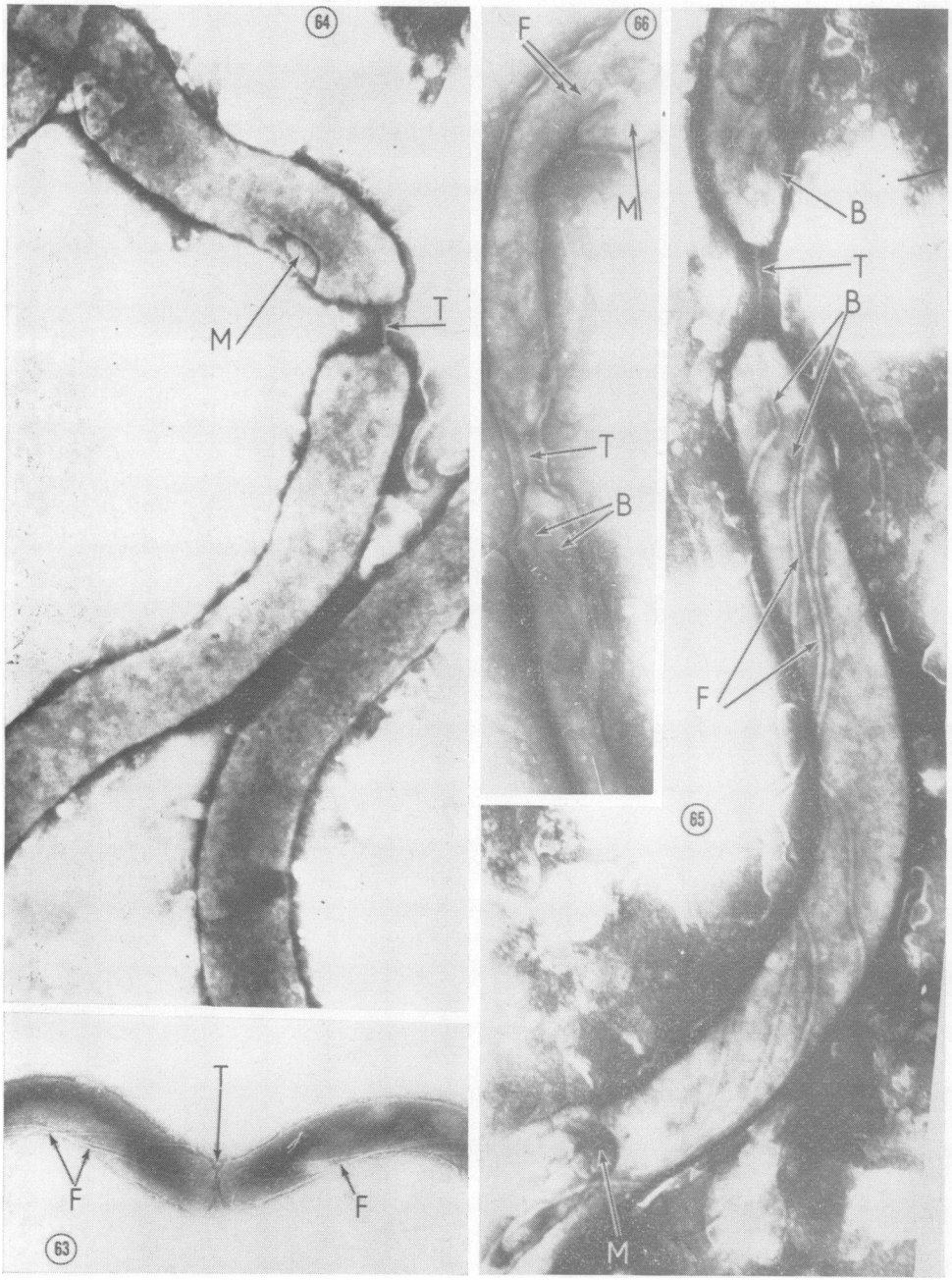
FIG. 58.—*Treponema pallidum*, strain 7. ×130,000. Structure of fibrils.



FIGS 59 and 59A.—*Treponema pallidum*, strain Stavropol 7. 14-day growth. ×36,000. Cryolysis. Differential centrifugation. Deep layer of fibrils visible.



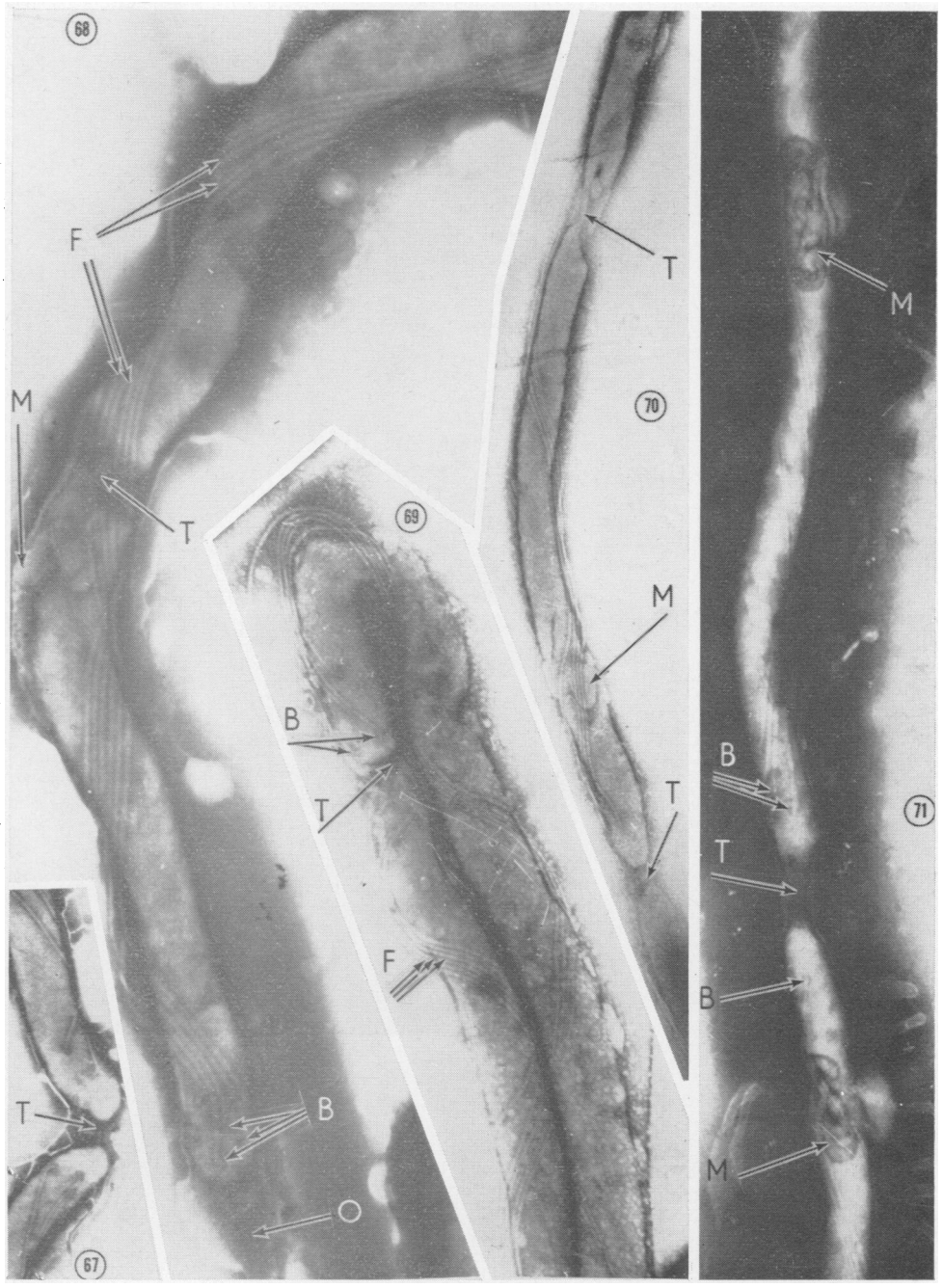
FIGS 60, 61, and 62.—*Treponema pallidum*, strain Stavropol 7. 7-day growth. ×27,000.



FIGS 63 and 64.—*Treponema pallidum*, strain 5. 7-day growth. ×27,000. Transverse fission of treponeme.

FIG. 65.—*Treponema pallidum*, strain Stavropol 7. 7-day growth. ×27,000. Transverse fission of treponeme, showing isthmus, new basal granules, and mesosomes.

FIGS 66 and 67.—*Treponema pallidum*, strain 5. 7-day growth. ×27,000. Transverse fission.



FIGS 68, 69, and 70.—Stavropol 7 strain. 7-day growth. ×27,000. Various stages in transverse fission.

In Figs 68 and 70, fission can be seen in the mesosome itself.

FIG. 71.—*Treponema pallidum*, strain 4. 15-day growth. ×27,000.

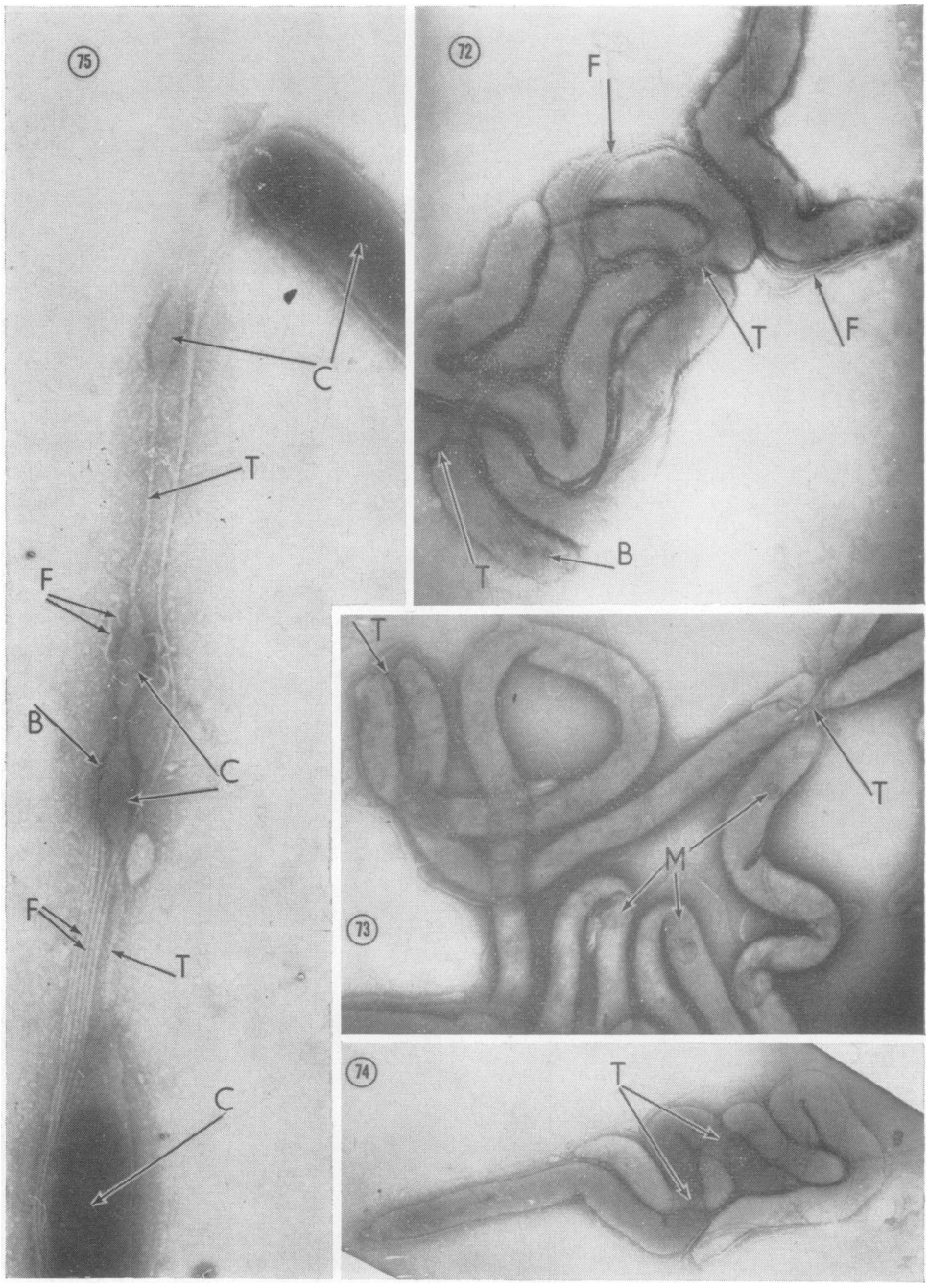


FIG. 72.—*Treponema pallidum*, strain 4. 15-day growth. ×27,000. Multiple fission.

FIGS 73 and 74.—*Treponema pallidum*, strain 5. 7-day growth. ×27,000. Multiple fission.

FIG. 75.—*Treponema pallidum*, strain Stavropol 7. 7-day growth. ×27,000. Multiple fission.

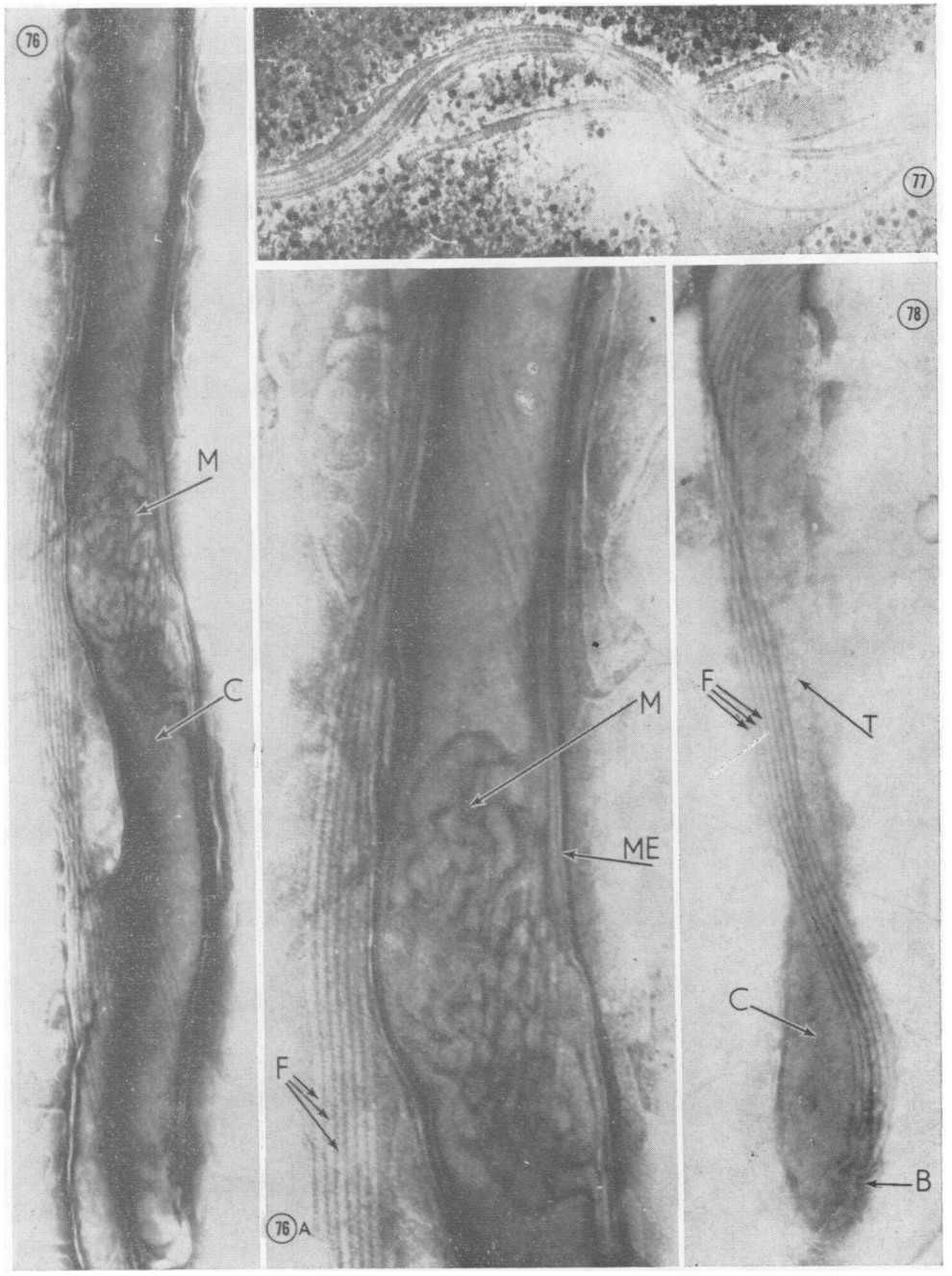
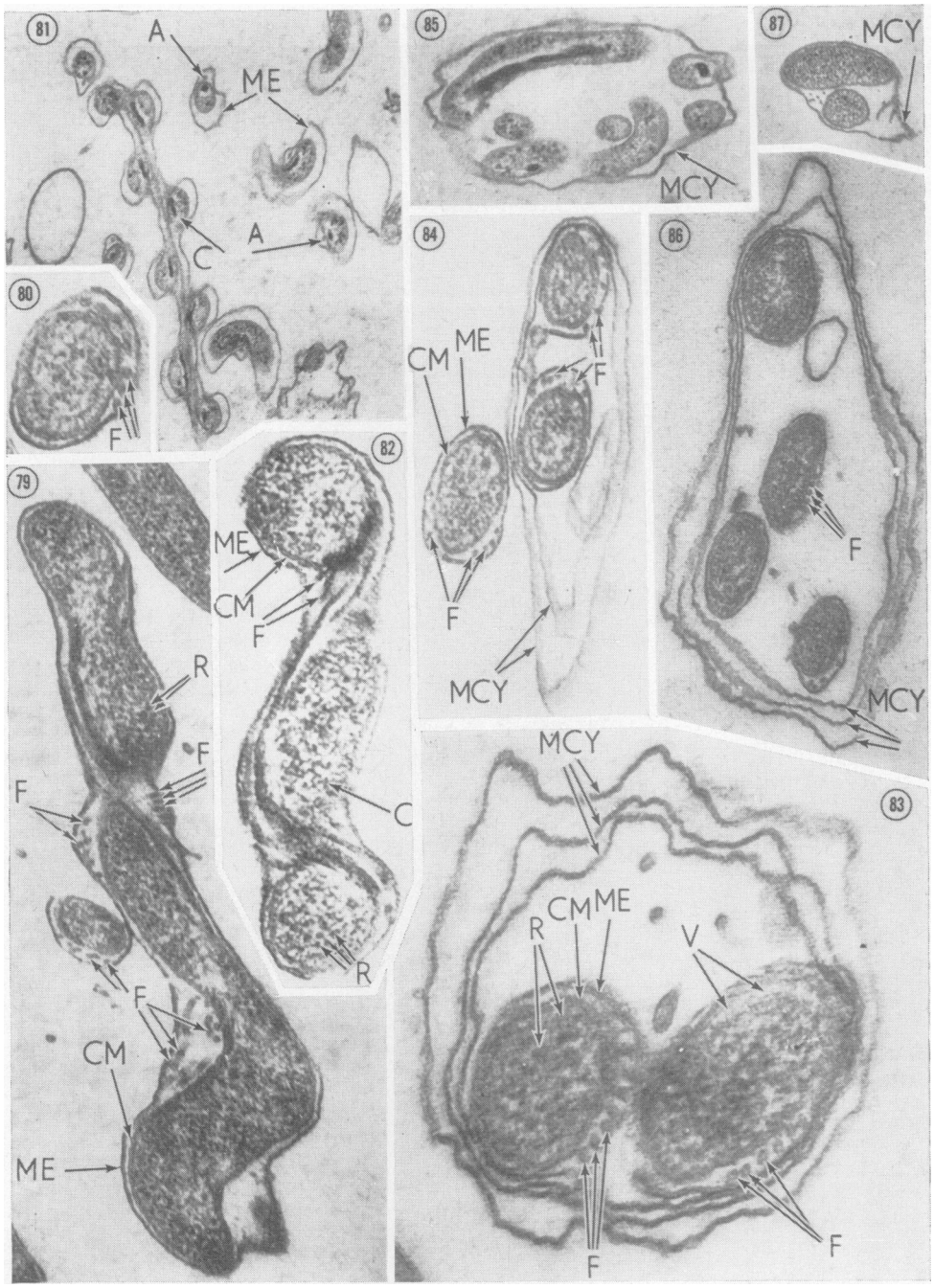


FIG. 76.—*Treponema pallidum*, strain Stavropol 7. 7-day growth. ×27,000. Fission of treponeme and participation of fibrils.
Fig. 76A shows a detail of mesosomal structure.

FIG. 77.—*Treponema pallidum*, strain Kazan 2. 14-day growth. ×36,000. Fibrillar structure.

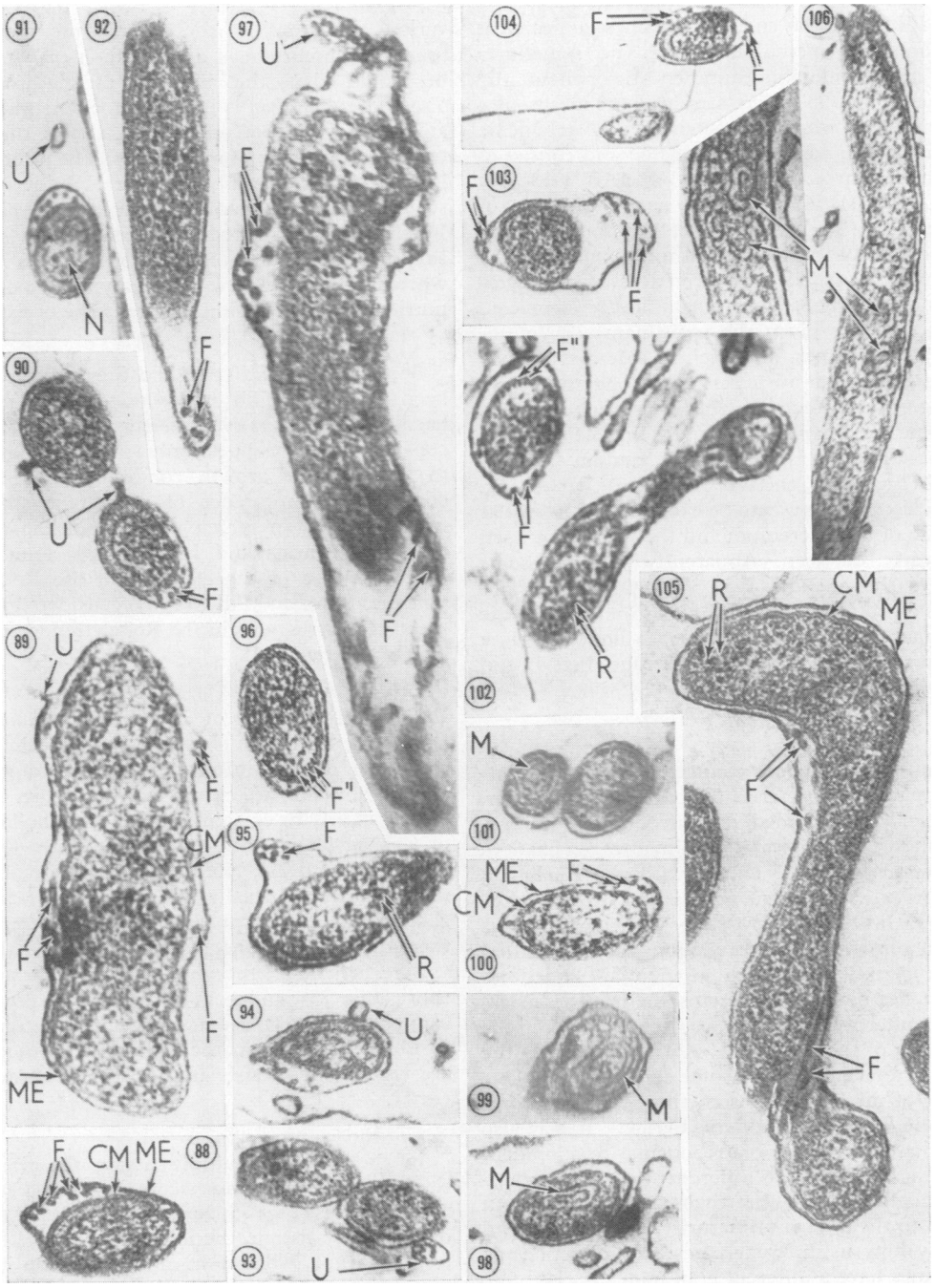
FIG. 78.—Stavropol 7. 7-day growth. ×27,000. Participation of fibrils in division.



FIGS 79 and 80.—*Treponema pallidum,* strain Kazan 2. ×27,000 Arrangement of fibrils and ribosomes in cytoplasm.

FIG. 81.—*Leptospira biflexa,* 21-day growth. ×36,000. Ultrathin section. Arrangement and course of axial filament and its relationship to envelopes and cytoplasm.

Figs 83, 84, 86, and 87 show ultrathin sections of a cyst of *Treponema pallidum.* In addition to the common envelope of the cyst (MCY), the structural features of the treponeme are clearly visible.



FIGS 82, 83, 84, and 86–106.—*Treponema pallidum*, Kazan 2 strain. 4-day growth. ×27,000.

FIG. 85.—*Leptospira biflexa*, 21-day growth. ×36,000.

Figs 89, 90, 91, 93, and 94 show the bundle of fibrils (U) situated outside the treponeme.

Figs 93 and 97 show individually cut fibrils in the bundle.

In Figs 98, 99, and 106 the structure of the mesosomes is visible.

Figs 103 and 104 show the arrangement of fibrils on both sides.

In addition to the small round mesosomes with or without an operculum, which are sometimes found in considerable numbers throughout the treponeme (Fig. 23), there are also large mesosomes occupying the whole diameter of the treponeme and even more, which are elongated and consist of a system of convoluted tubules (Figs 4AB, 30, 31, 32, 34, 39, 40, 41, 42, and 43) and sometimes of granules (Fig. 34). Opercula are also found on top of these. Both the small round mesosomes and the large oval structures are derivatives of the cytoplasmic membrane, being anatomically connected with it, and are given the same name of "mesosomes". Mesosomes (M) which lie side by side may be interconnected by means of a long thin canal (S) (Fig. 30). Sometimes the impression is given that some mesosomes are empty (Figs 41 and 65), and sometimes it is as if the operculum had just opened (Fig. 43W), the way had been cleared (Fig. 57), and something was to be excreted. The lamellar structure of the operculum and the mesosome itself can clearly be seen. Apparently the mesosome opens to the outside (Fig. 57V). On the surface there is a protuberance (Fig. 31Y), round which the fibrils make their way, but there is undoubtedly a way out under the external membrane (Figs 43 and 57).

Various functions are connected with the membrane systems of bacteria: fission, spore formation, exchange with the medium surrounding the cell, biosynthesis, the respiratory chain, nitrogen fixation, photosynthesis, and chemosynthesis (Gel'man, Lukojanova, and Ostrovskij, 1966). It must be assumed, however, that these various structures differ in construction and function, about which, as yet, we simply do not know enough. Sometimes fission takes place in the mesosome itself (Fig. 32) and the outer wall grows in, as it were, between the two halves of the mesosome. A bridge between the dividing treponemes can also be seen (Fig. 68). The number of mesosomes is sometimes very large but their type of structure differs (Fig. 32). It may also be that in the process of treponemal development mesosomes are found at different stages of development themselves and that their structure varies in relation to their stage of maturity. Figs 22, 24, and 32 show how in different parts of one treponeme mesosomes have a different appearance.

Murray (1963) considered that the membrane structures were not an obligatory structural feature in bacteria but represented a means of intensifying various functions of the cytoplasmic membrane that were important at a given moment. It should be noted that the number of mesosomes in *Treponema pallidum* is inconstant. Sometimes there are very few and at other times almost the whole treponeme consists of mesosomes. The same occurs in leptospirae. Possibly this corresponds to some stage in the development of *Treponema pallidum*. For instance, when there is intensified fission into small segments, the number of mesosomes is large, whereas there are few of them in a good fresh nutrient medium during the first stage of fission.

Some authors (Edwards and Stevens, 1963) have been unable to find mesosomes in the coliform bacillus, and others (van Iterson and Leene, 1964) consider that they appear only as a result of exposure to unfavourable growth conditions. Others again connect the appearance of membrane structures with a period in which the rate of biosynthesis increases. Kawata and Inoue (1964) found them after digestion of the cell wall of the Reiter treponeme. Ryter and Pillot (1963) considered that the membrane structures in the Reiter treponeme were poorly differentiated.

Transverse fission of *Treponema pallidum* may lead to the formation of two or more specimens. In the case of division into two, the new basal granules (B) and fibrils (F) are clearly marked and an isthmus (T) can clearly be seen in the shape of a hollow tube of varying length inside the outer walls (Figs 63, 64, 65, 69, and 71). Not far from the site of fission mesosomes are usually found. All this may be absent in the case of multiple fission. The treponeme then divides into short segments; the isthmuses (T) are very long, the young treponemes are very short (Fig. 75), and new fibrils and basal granules cannot be seen. In some treponemes the remnants of fibrils or fibrils in the course of forming again are visible. Mesosomes are not seen in these forms either.

In the case of multiple fission the fibrils are sometimes not cast and become elongated just like the isthmuses (Figs 75, 76, 76A, and 78). In the case of transverse fission, the isthmus takes the form of a hollow tube, whereas in multiple fission it seems to consist of fine individual filaments (Figs 60, 61, 62F″). Mesosomes take an active part in the process of fission (Figs 65, 66, 68, and 70). They are situated either not far from the site of fission (Fig. 71) or at the site itself (Fig. 68). Sometimes the treponemes become twisted and grow thinner and divide at

points of intersection (Figs 72, 73, and 74). Quite often there are many mesosomes in such treponemes (Fig. 73).

Interwoven and closely touching forms of treponemes are of considerable interest. One may be thin and another twice as thick (Fig. 45). One may be light in colour and the other dark (Figs 47 and 48). Finally, one may appear homogeneous and the other granular (Figs 44 and 46). It should be noted that the same phenomenon occurs in leptospirae (Figs 49 and 50). Perhaps one of them is a male organism and the other a female. Would it not be correct to assume the existence of a sexual process in treponemes? This is suggested not only by these and similar photographs but also by the fact that the sexual process in treponemes apparently takes place in the form of close contact along their whole length in such a way that the coils coincide closely.

In any case differences in thickness or in the granular or homogeneous nature of treponemes and leptospirae are not signs of degeneration. The granular structure of treponemes has a peculiar arrangement (Fig. 52). On the surface a fine bundle of fibrils can be seen (Fig. 52) and there is also a deeper fibrillar network (Fig. 51).

The means of insertion of the fibrils is still not clear. Pillot, Dupouey, and Ryter (1965) suggested that each fibril was attached to the ends of the treponeme. This is not quite accurate, and the question may be settled now in the following way. The fibrils attached to the basal granule at one end stretch towards the other end, but their other extremity is attached not to the opposite end of the treponeme but to a point somewhere on the treponemal body. Fig. 57 shows that fibrils may be attached with the help of basal granules even to the middle of the treponeme, but obviously this is not always the case. The fact that the fibrils attached to the different ends of the treponemes are not the same fibrils is confirmed also by the difference in numbers of fibrils in different parts of the treponeme. Thus, in Fig. 53, three fibrils may be seen on one end, ten in the middle, and so on. The same can be seen in some other figures.

The surface of the fibrils has the same structure as that of flagella. Valentine and Horne (1962) gave photographs of flagella of *Salmonella typhimurium* which showed a very similar structure to that of the treponemal fibrils given in our figures (Figs 58 and 77).

Flagella, like fibrils, consist of spherical subunits, tightly packed and spirally arranged along the axis of the flagellum. The central zone of the fibrils, like that of the flagella in photographs given in various publications, is filled with contrast substance.

It is also commonly believed that the treponeme winds round the bundle of fibrils. However, while this is quite likely in the case of leptospirae, the process is more complex in treponemes.

The arrangement of the fibrils has also not been elucidated. Cross-sections seem to show that they are situated underneath the outer wall, sometimes nearer to that wall, sometimes nearer to the cytoplasmic membrane and sometimes again entering the cytoplasm. In some sections no fibrils are visible (Figs 94, 101, and 105). There are also cases of fibrils being on the opposite side beneath the outer membrane. In these cases their numbers are identical (Fig. 103). In most cases, however, one fibril is on the other side (Fig. 100). The same cross-sections show that on the outside of the treponeme a round structure is quite often found (U) with osmophil granular inclusions (Figs 90, 91, 93, 94, 97, 98). Possibly this is a bundle of fibrils situated on the outside of the treponeme. In addition, if it is separated from the outer envelope by cryolysis, with subsequent separation by differential centrifugation in a discontinuous saccharose density gradient, in addition to the usual bundle of fibrils a huge number of thin fibres passing along the long axis of the treponeme can be seen. In places they cross each other when they go round to the opposite side (Figs 59 and 59A). They are roughly four or five times as thin as the external fibrils. So far it is not clear whether these thin fibres are a second layer of fibrils or whether the cytoplasm itself is a structure consisting of a system of slender fibres or tubes. There is more evidence in favour of the first supposition. On cross-sections (Fig. 102), under the cytoplasmic membrane at the level of the ordinary bundle of fibrils or on the opposite end, a number of alveoles with a cross-cut punctate structure in the centre can sometimes be clearly seen. Apparently this is indeed a second layer of more deep-lying fibrils (Figs 90, 96, and 102). In *Borrelia caucasica* there are many fibrils (ten to twelve) but they are much thinner than in *Treponema pallidum* (Fig. 55).

What is the significance of structures such as the basal granules and various types of fibrils detected

in the treponeme? Do the basal granules serve only as a point of insertion for the fibrils or as pivots for the movement of the fibrils attached to them? It must be assumed that they are not simply points of insertion. It is clear that movements are made with the help of the thick bundle of fibrils, but does this apply to all forms of movement characteristic of the treponeme or only to some of them, being effected by the thin interwoven threads on the treponeme? Or again, have they some other significance? For instance, they might possibly take part in nutrition. This might be assumed because they are quite often situated under the cytoplasmic membrane where there are fungiform cytoplasmic structures (Fig. 80), which some authors consider to be the site of the most active enzymatic processes in a number of bacteria (Birjuzova and Mejsel', 1964). It is not beyond the bounds of possibility that they play the role of a support for the cytoplasm—a skeleton as it were. As yet we do not know their precise purpose.

It is simpler to explain the tubular structure of the isthmus that joins dividing forms of treponeme. It is probably through the isthmus that nutrients are carried until separation is complete.

In cases of intensified fission, when the terminal structure has been shed and fission takes place at several sites, it is possible to see clearly along the isthmus, between the dividing specimens and the treponeme, regular rows of fibres and a system of tubes. Sometimes there are many such fibres (Figs 60, 61, and 62) and sometimes they correspond to the number of fibrils. Meanwhile, in a number of cases, it is possible to distinguish completely between the elongated fibrils and the isthmus (Figs 78, 54, and 62).

The outer wall at the level where the fibrils are situated sometimes rises well above the surface of the treponeme (Figs 95 and 97) and may give the impression of an undulating membrane. In the cytoplasmic substance itself large granules are encountered in addition to the small osmophilic granules. Both are ribosomes, in which protein synthesis takes place (Figs 82, 89, 95, 105). In places in which a mesosome is present in the section its structure consists of two rows of alveoles (Figs 99–106). Quite often it is also possible to see pathways in the mesosome entering the space between the outer wall and the cytoplasmic membrane.

We have already described the picture seen in longitudinal sections. At the present time we cannot refrain from merely illustrating once more the clear-cut two-layered structure of the outer wall and the cytoplasmic membrane (Fig. 105), the course and arrangement of the fibrils cut in various planes and at various levels in the treponeme (Figs 79, 82, 89, 97, and 105), the granular structure of the cytoplasm (ribosomes) and the presence of a small number of large osmophilic granules (polyribosomes) surrounded by a clear zone (Figs 82 and 105), and mesosomes taking the form of convoluted canals. The absence of granular structure in the cytoplasm in places where fibrils pass to the other side comes out clearly. Figs 89 and 90 point to the existence of an external bundle of fibres (U) or some sort of exit in the outer wall.

If longitudinal sections of *Treponema pallidum* and leptospirae are compared (Fig. 81), a great difference will be seen in the structure of the two micro-organisms; this difference also shows clearly on cross-section.

Despite some common features in their biology (particularly cyst formation), their structure is basically different.

In the present paper we shall not touch on granules or spore-like structures in treponemes but shall briefly discuss the cysts.

This problem has not been finally resolved. Some investigators do not accept that cysts are present in treponemes, while others consider them to be the result of degeneration. This is due to the fact that the process of cyst formation occurs in response to various unfavourable conditions. With lengthy exposure to unfavourable factors at a relatively low level of intensity, cysts are formed and resistance to the particular factor concerned increases. If the treponeme is exposed to very intense unfavourable factors, the cysts which have been formed die and disintegrate. We see in this also the reason for the wide divergence of our opinion from that of Pillot (1965) regarding cyst formation in treponemes. His experiments quite correctly indicate that cysts are formed under the influence of various factors. Ultrathin sections of cysts also clearly show signs of degeneration in the "spheroids" mentioned in his work. These are dead cysts. His paper, however, contains incorrect interpretations of his own results, since he groups all cysts together and considers them all to be degenerative forms. The motility of

the spheroids suggests that they are viable and their formation in response to various factors bears witness only to the general laws of cyst formation as a reaction to unfavourable conditions of existence and indicates that these are formed for defence and long-term survival.

In our earlier papers it was shown that cysts in treponemes and leptospirae, if treated by the negative-contrast method, have a protective envelope. If ultrathin sections of treponemes are examined, it is possible to see clearly all the structural features of the treponeme included under an envelope that is common to the cyst also and may have one or more layers (Figs 83, 84, 86, and 87 MCY). Some cysts contain round lamellar structures or formations filled with a granular mass. We suggest that this mass is a store of nutrient material. In addition, cysts are encountered which show signs of degeneration like those mentioned by Pillot (1965). Observation under the phase-contrast or dark-field microscope of material containing a large number of cysts shows that they are actively motile and are alive. In lengthy periods of observation treponemes can be seen issuing from the cysts. Finally, the seeding of material containing large numbers of cysts and almost no spiral treponemes on to fresh nutrient media with favourable conditions for growth leads to abundant growth of spiral forms.

Cysts are also found in cultivated treponemes, in pathogenic treponemes, in material from rabbits, and in leptospirae (Fig. 85).

All this has convinced us that the cysts are a way of ensuring defence and long-term survival for the treponemes.

In conclusion, we repeat that we are submitting factual material on morphology but that in regard to the functional significance of the structures described we lay no claim to precise interpretation. This significance must be elucidated by further comparison of electron-microscope results and experimental material with immunochemical and cytochemical research and comparative study of leptospirae, *Borrelia,* and spirochaetes, and of similar processes in other micro-organisms.

The study of this matter is a complex and protracted procedure. Persevering work is required by a number of different research teams equipped with modern precision apparatus.

Summary

Continuing their electron microscope studies of pathogenic and cultivated *Treponema pallidum,* leptospirae, and *Borrelia* with the use of ultrathin sections, the authors report a number of fresh findings. They describe a general homogeneous envelope or casing in *Treponema pallidum* and leptospirae. They found a difference in structure of the two ends of *Treponema pallidum* and their photographs show the structure of mesosomes, their arrangement and interrelationships, and their participation in the process of treponemal fission. In addition to the ordinary bundle of fibrils attached to the basal granules at the ends of the treponeme and along its length, they also describe a more deep-lying fibrillar network.

They reinforce with new results their former conclusions regarding the existence of cysts in *Treponema pallidum* and leptospirae, the presence of a general envelope, and the ultrafine structure characteristic of the unchanged treponeme. The motility of the cysts confirms that they are viable.

They illustrate the process of treponemal multiplication, the shedding of the terminal structures with their fibrils, and the structure of the fibrils.

Comparative photographs of treponemes, leptospirae, and *Borrelia* show the differences in their structure.

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Études additionnelles sur la morphologie du *T. pallidum* au microscope électronique

RÉSUMÉ

Continuant les études faites au microscope électronique sur des sections ultra-minces de *T. pallidum*, de leptospires et de *Borrelia* pathogènes ou de culture, les auteurs rapportent un nombre d'observations nouvelles. Ils décrivent une enveloppe homogène générale ou un revêtement chez le *T. pallidum* et les leptospires. Ils ont trouvé une différence dans la structure des deux bouts du *T. pallidum* et ont pris un certain nombre de photographies qui montrent la structure des mésosomes, leurs positions, leurs relations et leur participation au processus de fission du tréponème. En plus du faisceau ordinaire de fibrilles attachées aux granules de base aux deux bouts du tréponème et tout au long de sa longueur, ils décrivent aussi un lacis de fibrilles placé plus profondément.

Ils ont renforcé par leurs nouveaux résultats leurs conclusions précédentes à l'égard de l'existence de kystes dans le *T. pallidum* et les leptospires, la présence d'une envelope et la structure caractéristique ultra-fine du tréponème normal. La motilité des kystes confirme leur viabilité.

Par 106 photographies, ils illustrent le processus de multiplication des tréponèmes, la perte des structures terminales et leurs fibrilles et la structure des fibrilles. Les photographies comparées des tréponèmes, des leptospires et des *Borrelia* montrent les différences de leurs structures.